

THE GROWTH AND DIFFERENTIATION OF MACERATED EMBRYONIC DUCK KIDNEY TISSUE ON THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK

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ONE PLATE (THREE FIGURES)

INTRODUCTION

The results of heteroplastic transplantations usually lead to the conclusion that the host develops an antagonism against the transplant which inhibits its growth, and finally destroys the foreign tissue. Nevertheless, Hiraiwa ('27, '30) and Nicholas and Rudnick ('32) have shown that tissues of such widely separated forms as the rat and bird, can grow for a time in complete harmony during the embryonic stages. Ultimately, however, it seems as though either the host or donor, perhaps both, develop properties which make their tissues incompatible, and a destructive action occurs in which various cellular components of the host take part, e.g., connective tissue cells, lymphocytes, and granulocytes. The experiments considered in this paper were designed to determine to what capacity a macerated embryonic tissue, such as the kidney of the duck, would grow on the chorio-allantoic membrane of the chick, and to what extent the macerated condition of the tissue might influence the nature of the reaction of the host toward the transplant.

Murphy ('13, '14 a, '16, '26) transplanted hashed sarcomatous and embryonic tissue of the rat to the chorio-allantoic membrane of the chick. The tissue apparently grew well until the host reached 18 days of incubation, but between the

eighteenth day and hatching, the host reacted against the implanted tissue—a reaction which was indicated by an intensive lymphocytic infiltration. Murphy ('14 b) was also able to induce this lymphocytic reaction before the eighteenth day by grafts of adult chicken spleen on the chorio-allantoic membrane of the host in conjunction with the rat tissue.

On the other hand, Sandstrom ('32 a), in attempting to analyze the capacity of tissue for growth in heteroplastic transplants, found that intact pieces of undifferentiated metanephric tissue from duck embryos transplanted to the chorio-allantoic membrane of the chick would grow and differentiate into apparently normal kidney tissue, but that that which was already differentiated would not grow. Such tissue became necrotic and connective tissue invaded the transplants. In none of the grafts was a lymphocytic invasion noted, although Sandstrom ('32 c) has recently found infiltration of small lymphocytes in the early stages of the incorporation of the graft into the membrane. An attempt to induce a lymphocytic reaction against duck kidney tissue by the simultaneous presence of adult chicken spleen grafts (Sandstrom, '32 b) gave negative results.

At least two important factors might be considered in attempting to explain the apparent discrepancies in the work mentioned above. The first is the degree of relationship between host and donor. The rat, being more distantly related to the chick than is the duck, might incite a more intense reaction, and one of a different nature from that brought on by the duck. The work of Stevenson ('17 a and b), who transplanted sarcomatous tissue of the rat to the chick in conjunction with adult chicken spleen grafts, indicates that this is less important than one might suppose for his results showed no lymphocytic activity. The other factor is the condition of the tissue transplanted. Since Murphy used hashed embryonic rat tissue, and Sandstrom pieces of intact duck kidney tissue, the experiments reported in this paper were made in an attempt to determine whether the hashed form of the tissue might be partly responsible for the differences.

The results of this experiment have been reported in abstract form (Sandstrom and Kauer, J. T., '32).

MATERIAL AND METHODS

The hosts used in these experiments were embryos of single comb White Leghorn chickens; the donors, embryos and ducklings of White Pekin ducks. Throughout the experiment the hosts were incubated for a period of 9 days before the time of transplantation. The metanephric tissue was obtained from embryos of 13, 21, and 27 days' incubation, and from ducklings 1 week old. The method of chorio-allantoic grafting, except for necessary modifications, was essentially the same as that used by Willier ('24).

The metanephric tissue was removed and placed in a ground glass mortar with normal saline solution, kept at approximate 38°C., and ground with a glass pestle until no sizable particles remained in the solution. The macerated tissue was collected by centrifuging, and placed in a dish with normal salt solution kept at 38°C. It consisted of cell fragments, individual cells, small cell masses, and bits of tubules, which, because of the method of collecting by centrifuging, had no normal relationship with one another. Small portions, about 1 cu.mm. in size, were transplanted to the chick membrane. All the solutions and instruments used were sterilized, and the technique was done under fairly aseptic conditions. The grafts were removed at 24-hour intervals after an initial incubation period of 48 hours, and fixed in Zenker's solution. The tissue was embedded in paraffin, sectioned serially at 8 μ , and stained in Delafield's hematoxylin and azure-II eosin.

The condition of the tissue in the grafts obviously was dependent on the degree and nature of its incorporation into the chorio-allantoic membrane of the host. Therefore, only those grafts in which a direct vascular connection could be demonstrated, whether or not they were completely surrounded by the host membrane, were considered. Three of the thirty-eight transplants obtained were discarded for this reason.

Owing to the nature of the results obtained from transplanting the metanephric tissue from ducklings 7 days old, it was necessary to run a control for the experiment. It consisted of homoplastic transplantations of metanephric tissue from 5-day-old chicks made to the chorio-allantoic membrane of 9-day-old chick embryos. In this series the grafts were removed from the host 19 to 91 hours after transplantation. For the earlier stages of transplants, the results obtained by Sandstrom ('32 a and c) were used as controls.

EXPERIMENTAL DATA

1. Transplantation of macerated kidney tissue from 13-, 21-, and 27-day duck embryos

The macerated tissue from 13-day donors allowed to remain on the host membrane from 3 to 8 days showed progressive differentiation and organization as the time it remained on the host increased. For example, the grafts taken off on the third day (fig. 1) were practically all normal, with the exception of a few tubules at the periphery. The tissue, composed of many collecting and secreting tubules and a few renal corpuscles, appeared histologically identical to that found in grafts of intact duck kidney tissue of comparable age. However, the relationships of the various tubules were not normal. Extensive areas of undifferentiated metanephrogenous tissue were also present. The differentiation and organization of the tissue improved until the peak was reached in those grafts taken off on the seventh day (fig. 2). The number of tubules both collecting and secreting increased, as did the number of renal corpuscles. In sections, the structure seemed to approximate the organization of normal intact kidney. Actually, however, they never formed completely integrated structural units. When a few tubules were traced through their ramifications, it was found that practically every one ended blindly. Although most of the tubules consisted of many branches, the actual continuity of the various parts was not observed. Very little undifferentiated nephrogenous tissue remained.

In all the grafts from 13-day donors that were removed up to and including the seventh day, there was present, with but a single exception, a small amount of necrotic tissue which usually consisted of a few degenerated tubules at the periphery. The exception is that illustrated by figure 2, which was removed from the host on the seventh day, the age of the tissue of the graft being 20 days. Necrosis seemed to increase considerably by the eighth, ninth, and tenth days at which time the age of the transplanted tissue was 21, 22, and 23 days, respectively. In the grafts taken off on the eighth day, all of the tissue was necrotic, but that taken off on the ninth day, which will be considered later, did not have a great amount of necrosis. The tubules appeared normal. The transplant removed on the tenth day, however, was about two-thirds necrotic.

The antagonistic reaction of the host toward these transplants manifested itself by the infiltration of connective tissue and invasion of granulocytes. Infiltrations of lymphocytes never appeared. In the grafts removed up to and on the seventh day, there was very little connective tissue and granulocytes were never very abundant. After the seventh day, however, when the kidney tissue displayed considerable necrosis, connective tissue and granulocytes appeared more abundantly. The graft removed on the ninth day in which the kidney tissue present was for the most part normal, had an extensive invasion of connective tissue and granulocytes, indicating that the lack of necrotic tissue as compared to that present in grafts removed on the eighth and tenth days was due to the absorption of the necrotic area and subsequent replacement with connective tissue, and possibly to the presence of the granulocytes.

The results obtained from transplanting macerated duck kidney tissue of 21-day donors to chick hosts differed markedly from those just described for the younger tissue. The most obvious difference was the small percentage of normal kidney tissue found in any of the grafts (fig. 3). One of the grafts contained as much necrotic tissue as persisting kidney

tissue, but in all other cases, necrotic tissue composed either the greater part, or all, of the structure. The organization of the tubules was of a very low order. They were for the most part smaller in diameter than the tubules found in the first series. It cannot be definitely stated whether they were collecting or secreting tubules, or both. They appeared as collecting tubules of small diameter. Renal corpuscles were very scarce.

Another point of difference between this series and the one previously described consisted in the host reaction. While no differences were found in the results with respect to lymphocytes, the granulocytic invasion was very markedly increased, especially after the seventh day. The last grafts of the series, removed from the eighth to the eleventh days, contained dense collections of granulocytes. Another aspect of the host reaction was the connective tissue infiltration which was very marked. This connective tissue invaded the boundaries of the graft and usually isolated tubules or groups of tubules, thus breaking up the organization (fig. 3). Also contrary to the results obtained from younger donors, the incorporation of the grafts of this series was of a lower order generally, but all were vascularized by the blood vessels of the host.

The transplantation of tissue from 27-day duck donors yielded grafts very closely approximating those of the 21-day series. Kidney tissue was present in less than half of them, and where found, it consisted of only a few distorted tubules, the cells of which appeared normal but the diameters of which were considerably decreased. Renal corpuscles were very scarce. No lymphocytic invasion was present; and the granulocytic invasion, although quite marked, never reached the intensity that occurred in the 21-day series. Connective tissue was present in all of the grafts, and in some cases, had replaced much of the grafted tissue.

2. Transplantation of macerated kidney tissue from 1-week-old ducklings

The kidney of 1-week-old ducklings is without doubt completely differentiated and functional. When transplanted in the macerated condition to the chorio-allantoic membrane, it killed all of the twenty-five chick hosts within 48 hours. Because of the nature of these results, this series was repeated, and the hosts examined at the seventeenth hour of incubation. Out of the twenty-one hosts used all but six were dead. In these six exceptions the tissue was found to be completely unincorporated. This experiment was controlled by homoplastic transplants of macerated kidney from 5-day-old chicks made under identical conditions. The grafts were removed from the hosts at intervals between 19 and 91 hours of incubation. Out of the fifteen hosts used, only three were found dead.

DISCUSSION

The experiments demonstrate that macerated kidney tissue from duck embryos is capable of proliferating and differentiating when implanted on the chorio-allantoic membrane of the chick. They further demonstrate that the extent of this capacity and the degree of organization of the resulting grafts is dependent on the amount of differentiation of the donor tissue at the time of implantation. The macerated tissue from the younger donors, i.e., 13 days' incubation, was organized into grafts which appeared comparable to grafts of intact tissue of identical age, while that from older embryos of 21 and 27 days' incubation showed but little organization. Although some necrotic tissue was present in every graft, with one exception, the appearance of considerable areas did not occur until the age of the transplanted tissue reached 21 days. In general, these results are in accord with those obtained by Sandstrom ('32 a) who implanted intact kidney tissue of the duck to the chorio-allantoic membrane of both the chick and the duck. He suggested that a possible factor influencing the capacity of a transplanted tissue to grow successfully on the

chorio-allantoic membrane is its ability to function in the new environment. But because of the maceration of the tissue prior to implantation, the tissue in the grafts is not capable of organizing into a completely functional unit during the limited time it remains on the host. Instead, each graft must be looked upon as a group of separate units which are capable of differentiating independently. If, therefore, function is an important factor, the lack of functional integrity in the grafts might be expected to bring about earlier necrosis than in intact kidney tissue grafts. This was found to be true, for necrosis was found in practically all grafts. Additional evidence of the importance of function is found in the nature of the tubules in the grafts removed after the tissue was 21 days of age. The ratio of the number of apparently collecting tubules to the number of secreting tubules was noted to be markedly lower than the ratio found in grafts removed before that time. This is in accord with the suggestion made by Sandstrom (*loc. cit.*) who stated (p. 370) that "... if the degeneration of the kidney is correlated with its secretory function, then the secreting tubules should degenerate first." It is suggested that since the first marked evidence of necrosis and the first appearance of the decreased number of secreting tubules is found in the grafts removed after the total age had reached 21 days, perhaps the kidney of the duck is partly functional at this time.

The reaction of the host was manifested by the invasion of connective tissue and the infiltration of granulocytes. With the onset of necrosis these elements in the graft increased, and their intensity seemed to vary directly with the time the graft was allowed to remain on the host and not with the age of the host. Connective tissue infiltration was reported by Murphy ('14 a, '26) in grafts of hashed rat tissue removed after the host had reached the age of 18 days' incubation, and also by Sandstrom (*loc. cit.*) in grafts of intact duck kidney tissue transplanted to the chick membrane. The latter investigator found the connective tissue present in all grafts of donor tissue 19 days' of age and over, at which time

necrotic tissue was present. Neither Murphy nor Sandstrom reported an increase of granulocytes, and in this respect the results here recorded are more in accord with the results of Stevenson ('17 a and b), who found granulocytes appearing in large numbers in grafts of rat tumor tissue transplanted to the chorio-allantois of the chick. It is of interest to note that in none of the transplants of macerated duck kidney tissue grafted to the chick membrane did an infiltration of lymphocytes occur. In this particular, the results differ from those of Murphy (*loc. cit.*), who found an intense lymphocytic infiltration occurred in the grafts after the host had been incubated 18 days or more. Sandstrom ('32 a and b) reports no lymphocytic infiltration, but in a more recent report ('32 c) he states that lymphocytes were present in the early stages of incorporation of intact tissue grafts regardless of the age of the host.

Of special interest are the results of the implantation of macerated kidney tissue from 1-week-old ducklings to the chick membrane, which caused a 100 per cent mortality among the hosts within 48 hours. The outcome seems of considerable significance inasmuch as homoplastic implants of macerated kidney tissue from hatched chicks made to the chick caused only a small mortality which could easily be attributed to either experimental technique or to lack of virility of the host. Furthermore, Sandstrom ('32 a and b) obtained grafts from transplants of intact kidney tissue from ducklings, and even adults, when made to the chorio-allantoic membrane of the chick. The high mortality rate was probably due to the presence of anaphylactoid shock. This type of protein poisoning reviewed by Hanzlik ('24), is induced by the first injection of the foreign material. Among other effects, all of which are fatal to the host, there is a circulatory collapse. Although no experiments were made to check up the effect of the macerated duckling tissue on the chick host, it seems highly probable that a circulatory collapse of the chorio-allantoic blood vessels caused the asphyxiation of the chick host. Further investigation on this point is planned. However, the results

in general indicate that the protoplasm of the tissue released from the cell boundaries by maceration, is species specific. Just when this specificity appears in the developing tissue has not been ascertained. More than likely, however, it develops gradually. The mortality rate in the 27-day donor series, because of the relatively small number of hosts used, can be regarded only as suggestive. For the entire series it was 66 per cent; but 81 per cent of the mortality occurred in hosts that were opened 5 days after the transplantations were made. If this observation is indicative of the appearance of a species specificity in the tissue, it may perhaps be suggested that there is a progressive development, which does not manifest itself in grafts of intact tissue.

The fact that macerated embryonic kidney tissue can proliferate and differentiate on the chorio-allantoic membrane to form grafts of a high degree of organization, raises an interesting point, namely, whether or not embryonic kidney tissue, as well as other embryonic tissues, has the capacity to regenerate to form completely integrated structures much as in lower animals. For example, Child ('28) macerated *Corymorpha* and found that the cells, after dedifferentiation, reconstituted themselves into aggregates which redifferentiated to give rise to hydroid forms. Although this power of regeneration is lost, or considerably limited, in the tissues of adults of the more advanced organisms, it is possible that the tissues in the very early stages of development might retain the capacity to reconstitute themselves after the cells had been artificially separated. In a further investigation of the problem, it would be advisable to grind the tissue in sand to obtain a more complete maceration.

SUMMARY

1. This paper is concerned with the growth and differentiation of macerated embryonic duck kidney tissue transplanted to the chorio-allantoic membrane of the chick.
2. The results show that the macerated kidney tissue from younger donors, i.e., duck embryos of 13 days' incubation, was

capable of considerable proliferation and a high degree of organization. The macerated tissue from donor embryos of 21 and 27 days' incubation, however, was not capable of much proliferation or of a high order of organization.

3. The antagonistic reaction of the host toward the transplants manifested itself by the invasion of connective tissue and granulocytes. With the onset of necrosis these elements in the grafts increased, and their intensity seemed to vary directly with the time the graft was allowed to remain on the host and not with the age of the host. In none of the grafts was a lymphocytic infiltration observed.

4. The early appearance of necrosis in the grafts is considered an indication of the onset of function in the kidney tissue.

5. The transplantation of macerated kidney tissue from 1-week-old ducklings caused the death of every host within 48 hours. The death of the chicks was probably due to the presence of an anaphylactoid shock. The appearance of this reaction is interpreted to mean that species specificity has become definitely established in the duckling by this time.

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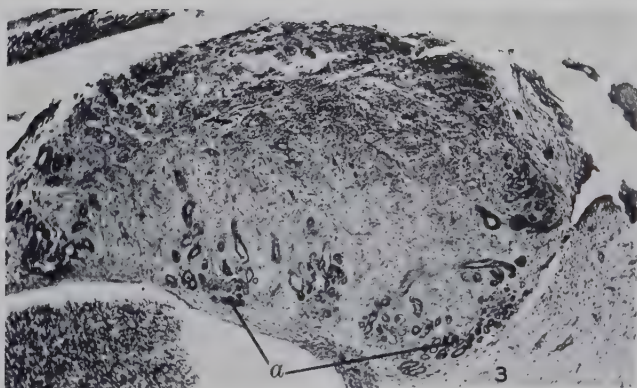
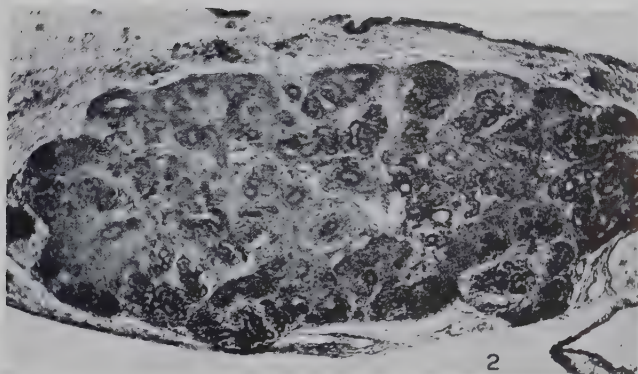
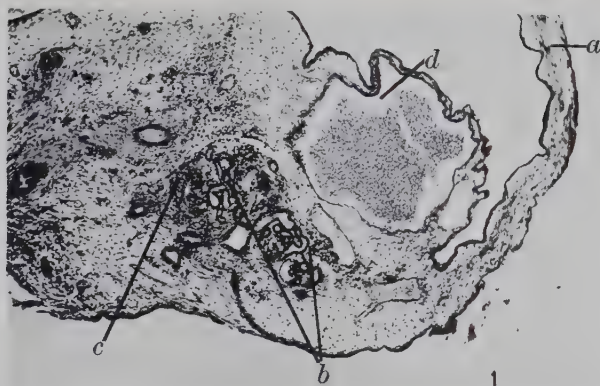
PLATE 1

EXPLANATION OF FIGURES

1 Section of a graft of macerated duck kidney tissue from a 13-day donor, after having grown on the chorio-allantoic membrane *a* of the chick for 3 days. Note normal collecting tubules *b*, and undifferentiated metanephrogenous tissue *c*. Chorio-allantoic blood vessel *d*. $\times 40$.

2 Section of a graft of macerated duck kidney tissue from a 13-day donor, after having grown on the chorio-allantoic membrane of the chick for 7 days. Note numerous normal tubules, both collecting and secreting. This graft shows the peak of differentiation and organization reached by the grafts in this experiment. $\times 40$.

3 Section of a graft of macerated duck kidney tissue from a 21-day donor, after having grown on the chorio-allantoic membrane of the chick for 5 days. Note extensive necrosis, degenerated tubules *a* and connective tissue invasion which isolated the tubules. $\times 40$.



HETEROPLASTIC TRANSPLANTS OF DUCK CARTILAGE TO THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK

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ONE PLATE (TWO FIGURES)

INTRODUCTION

The experiments reported upon in this paper were undertaken in an attempt to determine whether cartilage and bone of the duck is capable of unaffected growth when transplanted to the chorio-allantoic membrane of the chick. Sandstrom ('32) reported the results of heteroplastic transplants of duck kidney tissue to the chorio-allantoic membrane of the chick and duck, and suggested that the failure of the kidney tissue to continue growth after differentiation is probably due to its inability to function properly. Therefore, unless species specificity is an important factor, tissues such as cartilage and bone, for example, because of the nature of their functions, should be capable of differentiation and continued growth in heteroplastic chorio-allantoic grafts. The experiments reported in this paper, a preliminary report of which has been made in abstract form (Sandstrom and Kauer, G. L., '32) were made with these possibilities in mind.

That cartilage and bone grow and differentiate in homoplastic chorio-allantoic grafts has been noted by many workers. Danchakoff ('24) found that normal bone was formed in such grafts when parts of the femur anlage of chick embryos were allowed to grow on the host membrane. Murray ('28) obtained normal skeletal parts in grafts obtained from transplants of limb primordia of 2-day-old chick

embryos. Hunt ('32) reported normal cartilaginous and bony skeletal structure in grafts obtained from transplants of limb primordia of chick embryos ranging from 2 to 7 days of incubation. More recently, Studitsky ('32) reported normal bone formation from the transplantation of tibial bone.

Little work has been done on heteroplastic chorio-allantoic grafts of cartilage and bone. Hiraiwa ('27, '30) transplanted portions of 11-day rat embryos to the chorio-allantoic membrane of the chick. He obtained apparently normal cartilage and bone formation in the grafts after they had remained on the host for 8 days.

MATERIAL AND METHOD

The method of chorio-allantoic grafting first described by Rous and Murphy ('12) and subsequently improved by Willier ('24), was used in these experiments. The donor tissue obtained from White Pekin duck embryos of 6, 8, 12, 16, and 20 days' incubation, was implanted on the chorio-allantoic membrane of 9-day embryos of single comb White Leghorn chickens. The cartilage of either the femur or tibia of the donor embryos was removed, and after all mesenchyme had been teased from the perichondrium it was cut approximately into 1 mm. cubes. A piece of cartilage was then placed on or very near the blood vessels of the host membrane. After 9 days of further incubation the grafts were removed. A few were fixed in Zenker's solution and the remainder retransplanted to the chorio-allantoic membrane of new hosts. After an additional 9 days of incubation, they were again retransplanted and allowed to incubate for another period of 9 days. Thus some of the grafts remained on chick membrane for a total of 27 days. Since the age of the donor at the time of transplantation varied from 6 to 20 days, the total age of the duck tissue at the time of removal varied from 15 to 47 days. The grafts were embedded in paraffin, sectioned serially at 8μ , and stained with Delafield's hematoxylin and azure-II eosin.

DESCRIPTION OF THE GRAFTS

The general results of these experiments which are capable of tabulation are incorporated in the following table:

TABLE 1

Grafts of duck cartilage to the chorio-allantoic membrane of the chick

GRAFT NUMBER	AGE OF DONOR (DAYS)	TIME ON HOST MEMBRANE (DAYS)	TOTAL AGE (DAYS)	DERIVATION OF CARTILAGE	CARTILAGE	BONE	NECROTIC TISSUE	GRANULOCYTES	LYMPHOCYTES	REMARKS
404-5	6	9	15	Thoracic vertebrae	+	—	—	+	—	
404-16	6	9	15	Right wing	+	+	—	+	—	
403-3	8	9	17	Tibia	+	—	—	—	—	1
400-2	12	9	21	Distal $\frac{1}{4}$ tibia	+	—	—	+	—	
400-3	12	9	21	Medial $\frac{1}{4}$ tibia	+	+	—	+	—	
401-2	16	9	25	Prox. $\frac{1}{2}$ humerus	+	+	—	+	—	2
401-3	16	9	25	Distal $\frac{1}{4}$ tibia	+	+	—	+	—	
401-6	16	9	25	Prox. $\frac{1}{4}$ tibia	+	+	—	+	—	1
401-7	16	9	25	Distal $\frac{1}{2}$ radius	+	+	—	+	—	
401-9	16	9	25	Prox. $\frac{1}{8}$ humerus	+	+	—	+	—	
401-15	16	9	25	Prox. $\frac{1}{8}$ tibia	+	+	—	+	—	
401-20	16	9	25	Medial $\frac{1}{8}$ metatarsal	+	+	—	+	—	2
404-1	6	27	33	Prox. part femur	+	—	+	+	—	
404-2	6	27	33	Prox. part femur	+	+	+	+	—	2
403-2	8	27	35	Tibia	—	+	+	+	—	2
403-5	8	27	35	Tibia	+	+	—	+	—	2
403-7	8	27	35	Tibia	+	+	—	+	—	2
403-10	8	27	35	Tibia	+	+	+	+	—	2
400-4	12	27	39	Prox. $\frac{1}{4}$ tibia	+	+	+	+	—	
400-5	12	27	39	Prox. $\frac{1}{4}$ tibia	+	—	—	+	—	
400-7	12	27	39	Distal $\frac{1}{2}$ tibia	+	+	—	+	+	
400-8	12	27	39	Distal $\frac{1}{2}$ tibia	—	+	—	+	—	
400-10	12	27	39	Medial $\frac{1}{4}$ tibia	+	+	—	—	—	
400-12	12	27	39	Medial $\frac{1}{4}$ tibia	+	+	+	+	+	2
401-1	16	27	43	Distal $\frac{1}{8}$ femur	+	+	+	+	+	
402-4	20	27	47	End of femur	+	+	+	—	—	
402-5	20	27	47	End of femur	+	—	—	—	—	
402-7	20	27	47	End of femur	+	—	—	+	—	

1 = removed without allantois. 2 = connective tissue invasion.

Cartilage was found in the grafts obtained from 6-day donors which had remained on the host membrane for 9 days, while bone occurred in only one. The cartilage found in graft 404-5 was young and calcified with a deep-staining matrix, and was surrounded by perichondrium. This graft, which was well vascularized, showed no degeneration or necrosis. Few granulocytes and no lymphocytes were present. In graft 404-16 both cartilage and bone were present. The cartilage was of two types and represented normal differentiation and development. At one end of the graft only typical hyaline cartilage with a lightly-staining matrix was present, surrounded by perichondrium (fig. 1). Toward the other end the matrix was calcified, and at the extremity of the graft, calcified cartilage enclosing dendritic cells was surrounded by bone which was contiguous with the cartilage matrix (fig. 2). Thus all the typical stages of normal cartilage prior to and accompanying bone formation were found in this graft. Osteoblasts were present in the bone spaces closely investing the osseous spicules. Myeloid tissue was present from which the few granulocytes noted were derived. Here, as in grafts to be described subsequently, the granulocytes were formed, not as a reaction of the host, but from the myeloid tissue of the graft. Neither necrotic tissue nor connective invasion occurred in the graft; no lymphocytes were observed.

Only one graft of 8-day cartilage grown on the host membrane 9 days, was obtained. In this graft, which was fixed without the attached chorio-allantoic membrane, normal hyaline and calcified cartilage, but no bone, was present. A complete absence of necrosis, lymphocytes, and granulocytes was noted.

The two cases of 12-day transplants, the total age of each of which was 21 days, were obtained from adjacent portions of the same donor tissue. In graft 400-2, which was derived from the distal quarter of a right tibial cartilage, only normal hyaline cartilage was present. Graft 400-3 obtained from the shaft of the same cartilage, contained both cartilage and bone. The cartilage in this case was mostly calcified, containing

dendritic cells within the lacunae, although toward one end of the graft hyaline cartilage was present. Some bone occurred peripherally to the calcified cartilage, but no myeloid tissue was observed. Few granulocytes were present and no lymphocytes were noted. There was a complete absence of necrotic tissue. By considering both grafts, it may be seen that the development and differentiation of the cartilage in these transplants was in every way normal and reflected the condition present in a duck embryo of the same age.

In each graft, the total age of which was 25 days at the time of removal, both bone and cartilage were found in varying amounts, seemingly dependent upon the origin of the donor cartilage. All of the grafts contained calcified cartilage but the amount and character in each differed. Bone, usually peripheral, was present in all cases. In graft 401-2, the calcified cartilage contained dendritic cells which normally accompany bone formation. Within the bone spaces osteoblasts were noted. There was little myeloid tissue. Connective tissue invasion was observed about the bony portion of the transplant. Graft 401-3 also contained calcified cartilage and dendritic cells; the bone in this graft was less extensive than in graft 401-2, which may be explained by the derivation of this transplant. Osteoblasts and granulocytes were more numerous here than in the preceding graft. In graft 401-6, derived from the proximal quarter of a tibial cartilage, calcified cartilage containing normal cells was abundant. In the region of the relatively small amount of peripheral bone, dendritic cartilage cells were present within a calcified matrix. Few osteoblasts were found and no myeloid tissue was present. Graft 401-7 was typical of this series and contained dendritic cells and calcified cartilage completely surrounded by bone. Myeloid tissue was abundant. A relatively small amount of cartilage occurred in graft 401-9 but much bone was in evidence. Typical relationships of the bone and cartilage were found, the proportions of which may be explained by the derivation of the donor tissue. In graft 401-15, young calcified cartilage containing normal cells was

present at one end, while toward the other end, dendritic cells occurred. At this end, bone surrounded the cartilage and osteoblasts were present. Graft 401-20 was almost completely ossified with a slight amount of cartilage at the very center of the graft. Osteoblasts were abundant but no myeloid tissue was present; connective tissue filled the bone spaces and encapsulated the graft. This transplant was derived from the middle third of a metatarsal cartilage, which may account for the almost complete ossification found at 25 days. No necrosis was present in any of the grafts of this series and no lymphocytes were observed.

Calcified cartilage was found in both cases of 6-day grafts which grew on the host membrane 27 days; bone in only one, although both transplants were derived from contiguous portions of the limb anlage. After 18 days on host membrane, a rear limb graft containing cartilage and a bone shaft was cut into two pieces. The cartilage tip was retransplanted to 404-1, the bony shaft to 404-2. The different time of ossification of these portions, which occur normally in the differentiation of the femur, explains the occurrence of bone in only one of the grafts. The latter graft was markedly encapsulated and invaded by connective tissue. Poor vascularization accounted for the slight necrosis found in both grafts. No lymphocytes and few granulocytes were noted.

In the grafts of 35 days total age both cartilage and bone were again found in normal relationship. It may be noted that no cartilage appeared in one of the grafts, although the transplants were derived from right tibial cartilages. The irregular occurrence of bone or cartilage in grafts apparently obtained from identical levels of the original cartilages perhaps requires explanation. The derivation of the donor cartilage was not strictly defined by actual measurement, so that similar locations on the original cartilages as noted in the table must not be construed to refer to identical portions. Grafts 403-2 and 403-5 were derived from the same cartilage. Considerable myeloid tissue was present, giving rise to the granulocytes which were very numerous. Slight necrosis was

present in two of the grafts, and in all, connective tissue encapsulated the bone.

In the 39-day grafts, derived from 12-day donors, cartilage and bone were present in varying proportions seemingly dependent upon the derivation of the original cartilage. In grafts of the proximal or distal portions of the tibial cartilage, the amount of bone was very small and always peripheral. In grafts from the central shaft, bone predominated and cartilage, where present, contained degenerating cells. Granulocytes were more abundant in these grafts than in the preceding ones. A small number of lymphocytes was present in two of the grafts. Slight encapsulation of one of the transplants by connective tissue was observed.

In the single case of a 16-day graft on the host membrane 27 days, bone and cartilage were observed. Normal bone formation, with slight necrotic tissue, was present. No lymphocytes and a few granulocytes were noted.

In the oldest grafts, whose total age was 47 days, bone occurred in only one case, cartilage in all three. Hyaline and calcified cartilage were found in graft 402-5, bone in 402-4. This may probably be explained by noting the different regions of the cartilaginous end of the 20-day femur taken for transplantation. No lymphocytes were present in any case, few granulocytes in only one. Slight necrosis was present in the graft containing bone.

DISCUSSION

These experiments show that duck cartilage from donor embryos of 6 to 20 days of incubation grows and differentiates in a normal manner when transplanted to the chorio-allantoic membrane of the chick. All the normal manifestations of the development of cartilage are present, as are all the typical stages of its ossification resulting in normal bone formation in many of the grafts. The amount of bone present is probably determined by the derivation of the donor cartilage. Very little necrosis of either cartilage or bone tissue is found, even though the transplanted tissue at the time of removal was as old as 47 days, i.e., equivalent to a 20-day duckling.

The fact that cartilage will grow and bone will differentiate in grafts, even though they are heteroplastic, is not unusual; it has been reported in the literature on numerous occasions. Cartilage and bone, however, behave differently in transplants than do other tissues, in that they are capable of unaffected growth in a foreign environment for a considerable length of time. Hiraiwa ('27, '30), for example, found these tissues present in larger amounts than other tissues in grafts obtained from transplantations of sections of 11-day rat embryos to the chorio-allantoic membrane of the chick. In a preliminary paper, Hiraiwa and Willier ('27) commenting on this fact explained the persistence of cartilage and bone as due to 'differential survival.' The factors actually responsible for the survival, however, were not considered.

Sandstrom ('32, '33) in discussing the results of heteroplastic and homoplastic transplants of duck kidney tissue to the chorio-allantoic membrane of the chick and duck respectively, mentions two factors which influence the growth of tissues in chorio-allantoic grafts, namely, the rapidity with which the implant is incorporated into the host, and the ability of the grafted tissue to function properly after it has established itself. He found that the loosely bound structure of the metanephros of a 9-day duck embryo was readily incorporated into the host, and thereby, immediately vascularized. No necrosis appeared in the resulting graft before the onset of function, and no host reaction against the transplanted tissue occurred. Grafts from donors of older ages, in which the compactness of the organ interfered with incorporation and accompanying vascularization, elicited a decided reaction from the surrounding host tissue, and necrosis was not uncommon even before the onset of function. Furthermore, Sandstrom and Kauer ('33) found that necrosis in grafts of macerated duck kidney tissue made to the chorio-allantoic membrane of the chick was due entirely to the development of function in the various parts of the reorganizing tissue. Regardless of the age of the donor, the maceration had made the tissue comparable to that of the 9-day embryo and in this condition incorporation was rapidly effected.

Considering the above factors as obstacles which prevent the growth of transplanted tissues such as the kidney, and which must be overcome before normal growth can continue, it is of interest to note that neither exists when cartilage is transplanted. Cartilage is unique in that it is an avascular tissue. Regardless of whether the nutrient materials necessary for its survival permeate through the interstitial substance, or pass through a system of canaliculi, it does not depend upon complete vascularization in order to continue normal growth in grafts. It is sufficiently incorporated as soon as either the connective tissue of the host comes in contact with the surface of the cartilage or the blood vessels penetrate the perichondrium, if it is included also. Thus cartilage is incorporated into the host with great rapidity, and normal growth can continue from the first. Likewise, the second factor, i.e., the ability of the tissue to function properly after it is established, is no hindrance to the continued growth of transplanted cartilage. The function of cartilage is passive in contrast to the active function of such a tissue as kidney. Accordingly, it should grow for a considerable length of time once it is established. The present experiments give evidence in support of this suggestion, for duck cartilage is practically unaffected even though the tissue of the transplant is equivalent in age to a 20-day duckling.

On the other hand, bone tissue, although like cartilage in that its function is passive and can therefore continue growth once it is established in its new environment, differs from cartilage in that it requires complete vascularization for normal growth. The rapidity of incorporation is therefore an important factor when this tissue is transplanted. In none of the grafts removed after a period of 9 days on the chick membrane is there evidence of necrosis in the bone which differentiated from the transplanted cartilage. Although not extensive, it is found in some of the retransplants. The process of retransplantation is comparable to an initial transplantation. The bone in the retransplants, therefore, was subjected to conditions identical with those it would have en-

countered had it been transplanted directly from the donor. Thus, the necrosis in the grafts was undoubtedly due to poor vascularization during the early stages of the process of incorporation.

Another factor for the successful growth of any tissue in grafts made between different species might be species specificity. Any important reaction on the part of the host indicating resistance to a tissue from another species, that can be construed as an indication of species specificity, is entirely lacking in these experiments. However, though no direct evidence was obtained, it is not thought that species specificity is unimportant in heteroplastic transplantation of cartilage and bone. The results of Loeb ('25) and Loeb and Harter ('26) among many others, emphasize its importance. Loeb and Harter transplanting the xiphoid cartilage of the adult rat and rabbit to the guinea-pig, found that the perichondrium failed to proliferate, and that the grafts were invaded by lymphocytes. The transplanted tissue became necrotic and was invaded by connective tissue which gradually replaced the transplant. The authors are of the opinion that it would be only a question of time before the whole cartilage would be entirely replaced by connective tissue from the host. The results may be contrasted to those obtained earlier by Loeb ('25) who, working upon homoplastic transplantation of the xiphoid cartilage of adult rats, in which a species specificity factor was lacking, found that the cartilage would persist on the host for as long as 5 years. He remarks that if the conditions of experimentation were favorable, it might be possible to transplant adult cartilage serially for a long time and perhaps indefinitely.

Siebert ('31) studying heteroplastic transplants of cartilage between rats and guinea-pigs, found an increase in the intensity of the connective tissue reaction against the heterotransplants over that against the homoplastic transplants. The difference was apparent even in grafts of cartilage which had previously been killed by subjecting them to heat. It is difficult to correlate these latter observations of Siebert with

the concept offered by Loeb ('21) in which toxins (metabolic products) given off by the donor tissue are responsible for the host reaction against the transplanted tissue. The presence of connective tissue in and surrounding some of the grafts of the present experiments is not interpreted to be an indication of a host reaction against the heteroplastic tissue as such. By its very nature, the transplant even though normal and growing, acts as an inert foreign inclusion in the chorio-allantoic membrane, and therefore may be encapsulated. In no case is there noticeable an intensive, destructive action of connective tissue such as observed by Loeb and Harter (*loc. cit.*) which might be considered an expression of a species specificity either in the host or donor.

SUMMARY

1. These experiments are concerned with an attempt to determine whether tissue, such as cartilage and bone, is capable of unaffected growth when transplanted to the chorio-allantoic membrane.

2. Duck cartilage from donors 6, 8, 12, 16, and 20 days of incubation was used and allowed to remain on the host for 9 days. Retransplantation was effected in some cases to increase the length of time on the host to 27 days.

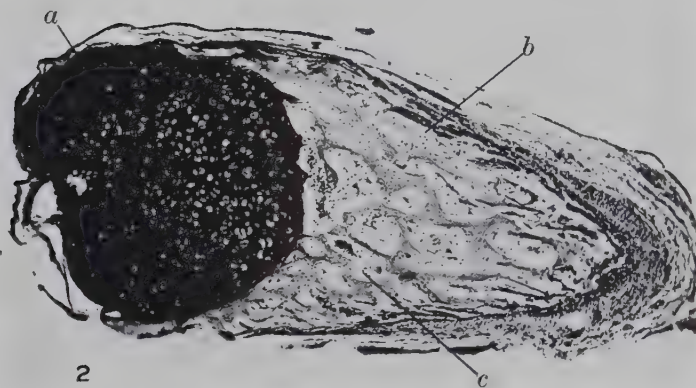
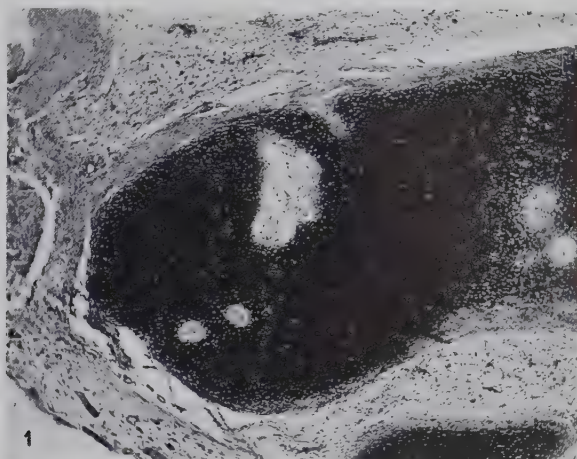
3. The results show that duck cartilage will grow and differentiate normally into bone when transplanted to the chorio-allantoic membrane of the chick. Very little necrosis of either cartilage or bone tissue is found, even though the age of the transplanted tissue at the time of removal ranged up to 47 days, i.e., equivalent to a 20-day duckling.

4. The longevity of cartilage in the grafts is due to its avascular structure which permits rapid incorporation, and to its passive function. Bone, too, grows for considerable lengths of time after it is transplanted. Its structure, however, prevents rapid incorporation which accounts for the small areas of necrosis in grafts of retransplanted bone.

5. No evidence of a reaction was observed which could be interpreted as an antagonism of the chick toward the duck tissue.

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1 Graft no. 404-16. Section of one end of graft of duck cartilage, from a donor 6 days of incubation, after having grown on the chorio-allantoic membrane of the chick for 9 days. $\times 40$.

2 Graft no. 404-16. Section of the opposite end of the graft shown in figure 1. Note the differentiation which has occurred at this end. Calcified cartilage *a* is partially surrounded by bone *b*, which contains myeloid tissue *c*. $\times 40$.

OBSERVATIONS ON THE DESCENT OF THE TESTES IN THE MACAQUE AND IN THE CHIMPANZEE

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TWO PLATES (EIGHT FIGURES)

In the human a number of pronounced changes have been observed in both the male and female reproductive tracts during late fetal and early postnatal life. These consist in the main of some form of enlargement of the reproductive tract followed by partial or complete involution immediately before or after birth. Here belongs, in the female, the marked hypertrophy of the uterus in late fetal life, followed by a pronounced postnatal decline, associated in a small percentage of cases with a brief period of actual uterine bleeding within a few days after birth. Here, too, belongs the rare occurrence of polycystic ovaries at birth, as well as the proliferation of the breasts in a small number of newborn, associated now and then with the actual secretion of 'witches milk.' In the realm of the male reproductive tract the hypertrophy of the prostate at term has been described. In the testes growth occurs until the eighth or ninth lunar month when regressive changes appear in the tubules. In the external genitalia, the scrotum is swollen and turgescient until a week or so after birth when this physiological tumescence declines. The details of these changes may be found in the writings of Halban ('04), Reiprich ('25), Neumann ('30) and Engle ('32). These changes had early been interpreted as due to some form of hormonal activity exerted at this period in the fetus, but with the recent discovery of two powerful hormones (theelin and prolan), circulating in the

blood of the mother and present in the fetus as well, renewed interest in the subject has been aroused. It is surmised that one or both of these hormonal substances is responsible for the observed changes. In keeping with such an interpretation is the fact that the two hormones, present in large quantities in the fetus at birth, disappear as a rule from its urine within a week after birth.

In view of these findings in the human, the writer considers it of value to report certain observations of a similar nature concerning the descent of the testes and changes occurring at birth in the scrotum of monkeys, more particularly of the macaque. Relatively little is known about the descent of the testes in monkeys, so that the peculiar changes observed in the macaque seem worthy of description.

To this are added for comparison some data collected on the descent of the testes in one of the great apes, the chimpanzee. The observations reported here form a part of a larger study to appear later upon the comparative anatomy of the genitalia of monkeys and apes. For the material used in this study I am indebted to Dr. Adolph H. Schultz, who placed his fine collection of embalmed monkeys at my disposal, and to Dr. Carl G. Hartman for specimens from his colony of macaques.

OBSERVATIONS ON MACAQUE MONKEYS (*MACACUS RHESUS*)

Persons working with young macaques are aware that during the juvenile period the testes of the macaque are undescended and that a scrotum, in so far as one is present at all, is represented by a flattened, transverse fold of loose skin stretching from groin to groin. Upon dissection of such juvenile macaques the testes are found to lie at or very near the inguinal ring. It is seldom possible to retract the testis into the abdomen, for although the inguinal ring is open, the orifice is too small to allow the testicle to return to the abdomen. The condition of the scrotum at this period is shown in figure 3 of an infant macaque of 212 mm. sitting height. From two living males I have the following notes.

In a male of several months of age the testes lie as small bodies just below the inguinal rings. Upon palpation they can be moved for a relatively short distance toward the scrotal folds. In a half-grown male, 'George,' one of Hartman's monkeys, the testes are inguinal in position, but they are easily movable by manipulation beneath the skin of the groin from the inguinal rings to the upper limit of the scrotal areas. The testes are about the size of robin's eggs.

From observations on healthy, developing macaques of the Carnegie colony, Hartman has informed me that descensus of the testes takes place into the scrotum at some period between the third and fifth years. For several years, however, at puberty the excursions of the testes are very great, the testes shifting readily from the scrotal sac to the inguinal canals. To quote experiences, a macaque monkey born in March, 1928, had on digital palpation the left testis in the scrotum, the right testis in the canal on January 9, 1931. In March, 1931, both testes were in the scrotum. However, a month later in April, 1931, the right testis was back again in the canal. Another macaque born May 15, 1928, had both testes in the canal on April 1, 1931, three years later. Similar observations, which he has kindly consented to my using, have been made by Dr. A. H. Schultz upon some of Hartman's monkeys. In one individual the right testis was in the scrotum, the left in the canal at 4 years, 4 months; however, at 4 years, 5 months both testes were in the canal, as was also the case at 4 years, 11 months. In another individual the left testis was in the scrotum, the right in the canal at 2 years, 10 months; at 3 years both testes were in the scrotum; nevertheless, at 3 years, 2 months both testes were back in the canal. In another, the testes were in the canal at 4 years. A monkey examined twice during the sixth and seventh years had scrotal testes on both occasions. During these years the flattened scrotal folds of the juvenile period become enlarged, filling out into pronounced scrotal sacs. Contrary to opinion in some quarters, the scrotum of the healthy adult macaque is a well-developed sac and by virtue of its anatomical char-

acter a true scrotum. The appearance of the normal adult scrotum is shown in figure 4. It should be recorded, however, that the testes of the adult macaque are at all times exceedingly mobile and can be readily pushed back by manipulation into the inguinal canal. They cannot be returned to the abdomen through the inguinal ring, although in my experience in dissecting three adults the inguinal rings were found to be open. The rings are, however, narrow slits no more than a millimeter or two in greatest diameter.

The course of events described so far would lead one to believe that the formation of the scrotum and the descent of the testes in the macaque were relatively delayed compared, for instance, with man. However, examination of late fetal stages and newborn macaques brings out something quite unexpected. I have had the opportunity of ascertaining the condition of the testes and scrotum in six fetal and three newborn macaques and much to my surprise have found that the scrotum instead of being inconspicuous and undeveloped is on the contrary very well developed. Indeed, instead of consisting of a mere inconspicuous fold of skin extending from groin to groin, it is swollen and oedematous, partaking of the shape and appearance of the adult scrotum. At first I thought this might be merely a postmortem artefact, but on finding the condition constant in the entire material I have been forced to conclude that this is the normal condition of the scrotum at birth. The typical appearance of the scrotum at this period is shown in figure 2 (fetus at term, sitting height 185 mm.). It will be observed that both scrotum and penis appear swollen.

Now even more unexpected than these findings is the fact that if the scrotum of the newborn or of a fetus near term is dissected, it is found that as a rule the testes have descended actually into or at least to the upper border of this swollen scrotal sac. Figure 5 illustrates the condition in a newborn macaque (sitting height 216 mm.). I have notes by Dr. A. H. Schultz regarding a fetus of *Macacus sinicus* with a sitting height of 157 mm., in which both testes are in the scrotum.

Furthermore, in a fetus of *Macacus rhesus* with a sitting height of 182 mm., the very small testes are also scrotal in position. My observations upon the location of the testes in fetal and newborn macaques are set forth in table 1.

The youngest macaque fetus which I have examined is a male from Hartman's colony with a sitting height of only 110 mm. and a gestation age of 75 days, hence near the middle of the gestation period. The genitalia of this fetus are shown

TABLE 1
Position of testes in fetal and newborn macaques

	SITTING HEIGHT	LOCATION OF TESTES
1. Fetus. <i>M. rhesus</i> no. 383	110 mm. 75 days gestation, Hartman	Testes in abdomen
2. Fetus. <i>M. sinicus</i>	157 mm.	Testes scrotal (Schultz)
3. Fetus. <i>M. rhesus</i>	182 mm.	Testes scrotal (Schultz)
4. Fetus 165. <i>M. rhesus</i>	182 mm.	Testes inguinal
5. Fetus 171. <i>M. rhesus</i>	185 mm.	Testes at upper border of scrotum
6. Fetus 270. <i>M. rhesus</i>	201 mm.	Right testis scrotal Left testis at upper border of scrotum
7. Term fetus. <i>M. rhesus</i> Aycock 1	191 mm.	Testes at upper border of scrotum
8. Stillborn. <i>M. rhesus</i> Hartman 1	216 mm.	Testes scrotal
9. Stillborn. <i>M. rhesus</i> Hartman 2	227 mm.	Testes scrotal
10. Infant 147. <i>M. rhesus</i>	212 mm.	Testes inguinal

in figure 1. The first point which this specimen brings out is that the scrotal anlage is well defined even at this early period. The scrotum is composed of two conspicuous eminences or hillocks. Upon dissecting the specimen the second point is brought out, namely, that the testes are still undescended and located at this period in the abdomen. Thus descent of the testes occurs in the second half of gestation, scrotal testes having been observed in fetuses of 157 and 182 mm.

The material at my disposal does not allow me to state precisely how many days after birth the scrotal swelling subsides or how soon the testes retract into the inguinal canal to assume the juvenile condition. The most I can say is that in an infant macaque of 212 mm. sitting height the genitalia have already assumed the juvenile configuration (fig. 3) with the testes located in the inguinal canal.

Summary

It appears, then, from these findings that the descent of the testes into a well-developed, swollen scrotum takes place frequently in the macaque during fetal life. This condition persists until shortly after birth when the testes ascend into the inguinal canal to remain there until the time of puberty. This ascensus is accompanied by a considerable degree of obliteration of the scrotum immediately after birth. At puberty the testes descend again to occupy the scrotum, the latter undergoing a marked enlargement.

OBSERVATIONS ON CHIMPANZEES

The unusual findings in the macaque naturally invite inquiry as to whether other Primates share a similar development. The data at my disposal for other groups allow me at the present time to give a complete answer for only one other representative, the chimpanzee. Here data are available which Dr. A. H. Schultz and I have assembled.

From these observations it is abundantly evident that the chimpanzee differs materially from the macaque, the testes descending permanently as a rule around the time of birth. Indeed, in a normal, well-preserved chimpanzee fetus of 193 mm. sitting height which I have dissected the small testes are completely descended into a well-defined scrotum. This fetus corresponds to a human one of the seventh or eighth lunar month. The genitalia of this fetus are shown in figure 7. Similarly in a chimpanzee prematurely born with a sitting height of 240 mm. Doctor Schultz has noted the presence of the testes in a scrotum which is quite large. Thus the chim-

panzee, one of the anthropoid apes, is identical with man and unlike the macaque in that the testes descend permanently around the time of birth. The chimpanzee has from before birth on a well-defined scrotum as illustrated by the drawings (figs. 7 and 8) of the genitalia of a fetus (193 mm.), and of a juvenile individual (416 mm. sitting height).

TABLE 2
Descent of testes in chimpanzee

SPECIMEN	SITTING HEIGHT	
Pan paniscus	193 mm. (fetus)	Testes in scrotum
Amer. Mus. Nat. Hist.		
Yerkes' specimen	240 mm. (premature)	Testes in scrotum
Phil. Zool. Garden	350 mm. (7 lbs.)	Testes in scrotum
	known age 6½ mos.	
McGregor Coll.	363 mm.	Testes in scrotum
Hopkins 262	384 mm. (8.7 lbs.)	Testes in scrotum
Hopkins 260	389 mm. (10.6 lbs.)	Testes in canal. Sick animal
Hopkins 315	416 mm.	Testes in canal
McGregor Coll.	429 mm.	Testes in scrotum
Hopkins 191	438 mm.	Testes in scrotum
McGregor Coll.	439 mm.	Testes in scrotum
Hopkins 217	447 mm.	Testes low in canal
Hopkins 215	463 mm. (19.5 lbs.)	Testes in scrotum
Hopkins 162	492 mm.	Testes in scrotum
Hopkins 297	477 mm.	Testes in scrotum
Hopkins 296	496 mm.	Testes in scrotum
Hopkins 372	510 mm.	Testes in canal. Very lean spec.
Dayton Nov. 1927	513 mm. (29.5 lbs.)	Testes in scrotum
Hopkins 290	515 mm.	Testes in scrotum
Hopkins 214	543 mm.	Testes in canal

DISCUSSION

The above outlined account sheds some light on several rather tangled and disjointed observations made previously by Bolk ('07) and by Mijsberg ('23). These writers hold in general that the catarrhine monkeys possess little or no scrotum and correspondingly little in the way of labia majora in the female. However, Bolk possessed six catarrhine fetuses, two males and four females, of which he was uncertain as to which genus they belonged. He believed that they

were either *Macacus* or *Cercopithecus* (*Lasiopyga*). At any rate he noted that in the two male fetuses the scrotum was well developed and that in the female fetuses there was a corresponding transverse swelling or fold between the thighs which he homologized with the scrotum of the males and looked upon, quite rightly, as the labia majora. The testes although descended into the inguinal canals had not reached the scrotum.

These findings Bolk looked upon as evidence that the catarrhine monkeys possess well-defined scrotal anlagen and labia majora during fetal life. In the absence of further adequate growth stages, more particularly full-grown healthy adults, he made the error of postulating that the scrotum and labia majora of catarrhines in the course of their postnatal development including adult life undergo partial or complete reduction. It was also natural for him to reach the phylogenetic conclusion that the ancestors of the recent catarrhine monkeys had more fully developed scrota than the present representatives. That his general conception is probably wrong is indicated by the present account of the development and fate of the scrotum and testes in the macaque. Before birth the testes frequently descend into the scrotum in the macaque; they ascend subsequently to remain in the inguinal canal until puberty when they descend again to lie permanently in a well-defined scrotum.

Concerning the fate of the labia majora I differ also with Bolk. In the newborn female macaque I find a transverse fold (fig. 6) similar to the one found by Bolk in female catarrhine fetuses, which I accept as the homologue of the scrotum. This structure practically disappears during the latter part of the juvenile period, but at puberty changes strikingly reminiscent of it appear again. In healthy females at puberty there develops a sharply demarcated, thickened area of skin, pigmented, puckered, and highly colored, which extends forward over the symphysis like a triangle, with its apex at the pudendal cleft. This area, according to Hartman ('28), is swollen at the time of puberty and has

underlying it, to either side of the mid-line, rounded masses of fat which can be rolled under the skin. These fatty masses are identical in character with similar masses in the original fold of skin present in early life. The area under discussion is moreover a specialized portion of that larger area around the genital orifice designated as the 'sexual skin.' Hartman ('28) adds that the swellings of puberty are usually confined to the region corresponding to the scrotum of the male and may well be homologized with it.

The second work on the subject of the descent of the testes in the Simiae that has to be considered is by Mijsberg ('23). This investigator dissected the genital tracts of a number of male monkeys. He says that in *Macacus* and *Semnopithecus* he found no scrotum and that the testes lay in a transverse fold of skin between abdomen and thighs. From this finding he generalizes as to the total absence of the scrotum in these species. Obviously Mijsberg was dealing with immature males. Indeed, in the majority of catarrhine monkeys, similarly to Bolk, this observer reports the absence of a scrotum and of scrotal testes. Moreover for one of the platyrrhine monkeys, *Ateles*, he observed a total absence of scrotum, while for the chimpanzee and orang he reports finding the testes incompletely descended. The present observations show clearly, on the contrary, that in the chimpanzee the scrotum is well developed and that the testes descend early, findings totally at variance with Mijsberg's single dissection.

The present investigator intends to report elsewhere upon the condition of the male genitalia in a series of platyrrhine and catarrhine monkeys. The evidence will show more extensively than the present report, limited to macaque and chimpanzee, that contrary to the findings of Mijsberg, healthy adult monkeys, both platyrrhines and catarrhines, have fully developed scrota occupied by descended testes. In *Ateles*, for instance, of which I have seen many adult males shot in the wild, the scrota are conspicuous and the testes fully descended. The tendency to consider the monkey as possessing a poorly developed scrotum and partially descended testes

arises, in my estimation, from the fact that the majority of investigators have dealt with small numbers of animals which have died in captivity, many of which have never attained functional sexual maturity. Mijsberg erects an hypothesis of the phylogeny of the scrotum in Primates. He postulates that the condition of descended testes with fully developed scrotum, as in man, is primitive, whereas complete or partial absence of scrotum and incompletely descended testes, as occur according to him in the majority of catarrhine monkeys and anthropoid apes, are more progressive, constituting a form of evolutionary reduction of the scrotum accompanied by a permanent ascensus of the testes. Since my observations do not substantiate his premises, I cannot accept his reasoning in regard to these phylogenetic matters.

Moreover, I cannot agree with the oft-cited quotation from Weber (*Die Säugetiere*, 1st and 2nd editions, '04, '28) to the effect that a scrotal anlage is lacking in most of the catarrhines, whereas it is found in a few of the platyrrhines. He cites specifically the absence of the scrotum in the Hylobatidae, whereas I have a series of specimens of gibbons which shows well-defined scrotal areas from fetal to adult life with scrotal testes present in juvenile individuals.

To return to the changes described in the male macaque, the present observations show that around the time of birth the scrotum is oedematous and swollen and that the testes are as a rule descended, but that a very short while after birth the testes ascend into the inguinal canal and the scrotum becomes reduced. This diminution of the male genital apparatus in the macaque at this period is in accord with the fact that changes of a similar, although not in all respects identical, character have been seen in the human. Here, too, the surmise seems justified that the changes encountered in the macaque scrotum and the ascent of the testes after birth are due to hormonal factors. It is of interest to examine to what extent this surmise is substantiated by facts. Unfortunately there has been no clear cut consensus of opinion as to whether theelin and prolactin occur during pregnancy in the

fetal and maternal circulations of macaques as they do in the human. As regards prolactin, Zondek ('31) reports the mouse test positive for pregnant macaques' urine; however, Allen, Maddux and Kennedy ('31) have recently reported negative results from injecting the urine of pregnant macaques into rats, and Snyder and Wislocki ('31) have reported negative ovulation tests in the rabbit. In reference to theelin, Allen, Maddux and Kennedy ('31) have recovered it from the placenta of macaques and from the urine of one pregnant animal.

Regardless of whether or not theelin and prolactin have been demonstrated in the pregnant macaque, what inferences can be drawn from other experiments, more particularly the results of administration of known hormones to immature macaques? In immature female macaques administration of theelin produces reddening and swelling of the sexual skin. Moreover, Saiki ('32) found that in immature animals pituitary injections produce reddening of the skin, which was interpreted as a sign that the ovaries had been induced to produce theelin. A similar result has not been obtained, however, in any female after injection of pregnancy urine extract (Engle, '32). Now swelling of the sexual skin in females might be assumed to be equivalent to swelling of the analogous structure, the scrotum in the male.

Hence the scrotal swelling in the newborn macaque might be interpreted as due possibly to the maternal sex hormone, theelin, were it not for some observations by Engle ('32). This investigator induced a descensus of the testes and scrotal swelling in juvenile macaque monkeys by injection of extracts of pregnancy urine, and to a lesser degree by anterior pituitary extracts. The picture of descensus of the testes and scrotal swelling obtained experimentally by Engle is identical in appearance with the physiological descent of the testes and scrotal turgor obtaining spontaneously at birth. These experiments indicate that anterior lobe hormone or the anterior lobe-like substance of urine is responsible for the changes. In spite of these findings it would be of interest to try theelin. It is quite possible that theelin might induce the scrotal swell-

ing without producing descent of the testes. Engle reports getting marked swelling from urine extract, whereas but little from anterior lobe extract. It is conceivable that even a slight residue of theelin remaining in urine extract might exert an influence of its own. Theelin itself when administered experimentally to normal male rats is reported to lead to grave injuries of the testes, whereas administration of fresh hypophyseal implants during the period of theelin treatment prevents the usual harmful effects of this substance on the male system (Moore and Price, '32). On the whole, Engle's experiments implicate the anterior lobe hormone or the anterior lobe-like substance found in pregnancy urine. Whether these extracts act by stimulating the production of theelin and whether theelin administered alone has an influence on the scrotum and the descent of the testes remain for further experiments to show.

From the identity of the picture Engle obtained, with the picture seen at birth, it would seem not unlikely that in both instances identical factors are operating. At any rate the condition found in the newborn macaque gives a further opportunity to approach the question of the hormonal factors experimentally. It is obvious that attempts should be made to prolong the birth condition by administration of hormonal extracts.

SUMMARY

1. The male macaque monkey exhibits at birth swelling and turgescence of the scrotum accompanied as a rule by the presence of descended testes. Shortly after birth the testes undergo ascensus into the inguinal canal while the scrotum regresses until it becomes a flattened fold of skin between the thighs. Not until the course of the third to fifth year do the testes descend again permanently at which time the scrotum again becomes fully formed.

2. In the chimpanzee the testes descend permanently as a rule before birth into the scrotum.

3. Well-defined scrotal Anlagen are present in the macaque and chimpanzee before birth, and throughout adult life the scrotum is well developed in normal, healthy animals.

4. The turgescence of the scrotum at birth and its subsequent regression accompanied by ascent of the testes can plausibly be attributed to hormonal factors. The phenomena in the male macaque at this period bear a resemblance to changes described in the human around birth. They also resemble very strikingly effects produced by Engle in young monkeys by administration of extracts of pregnancy urine or of anterior lobe.

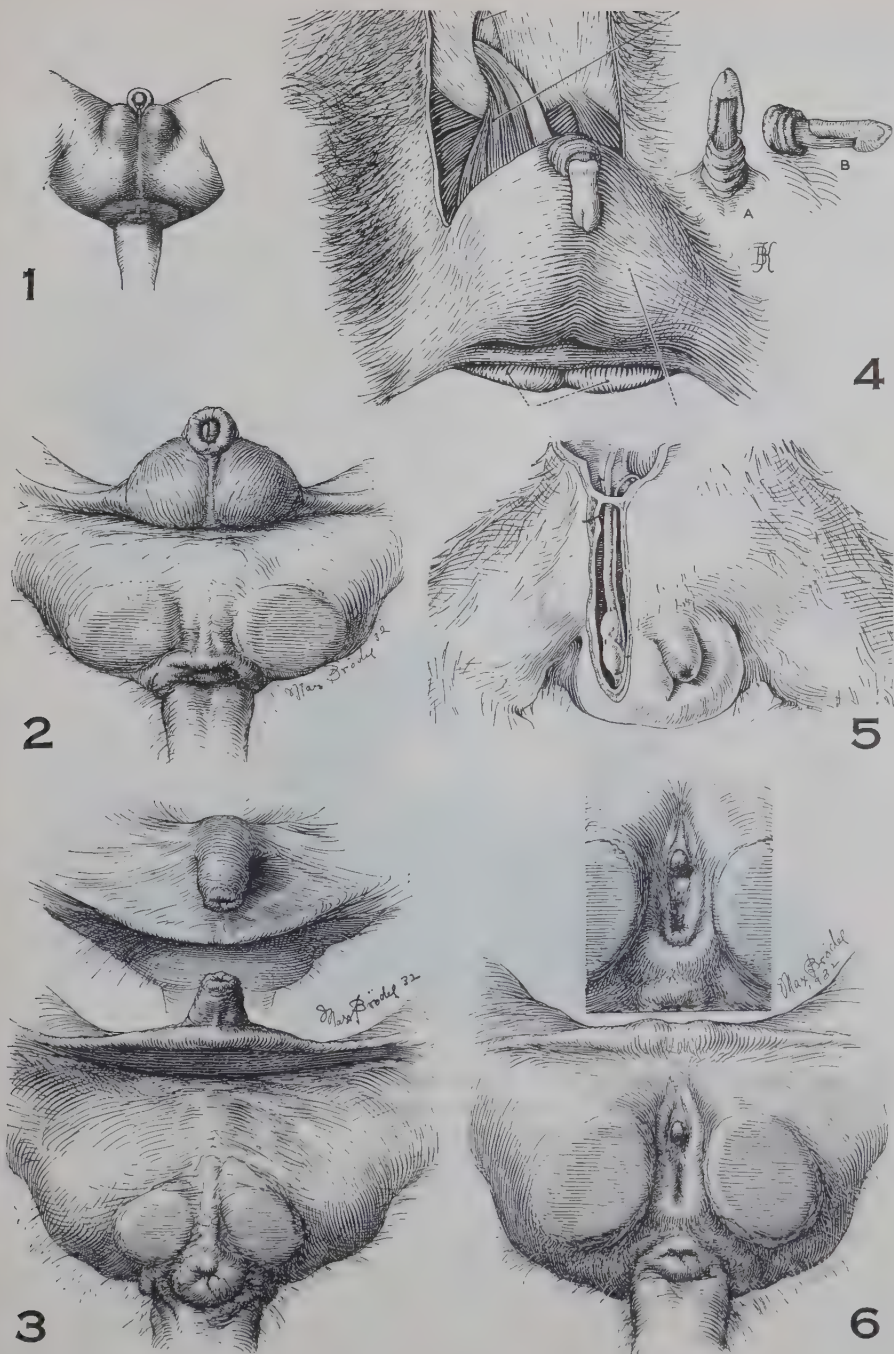
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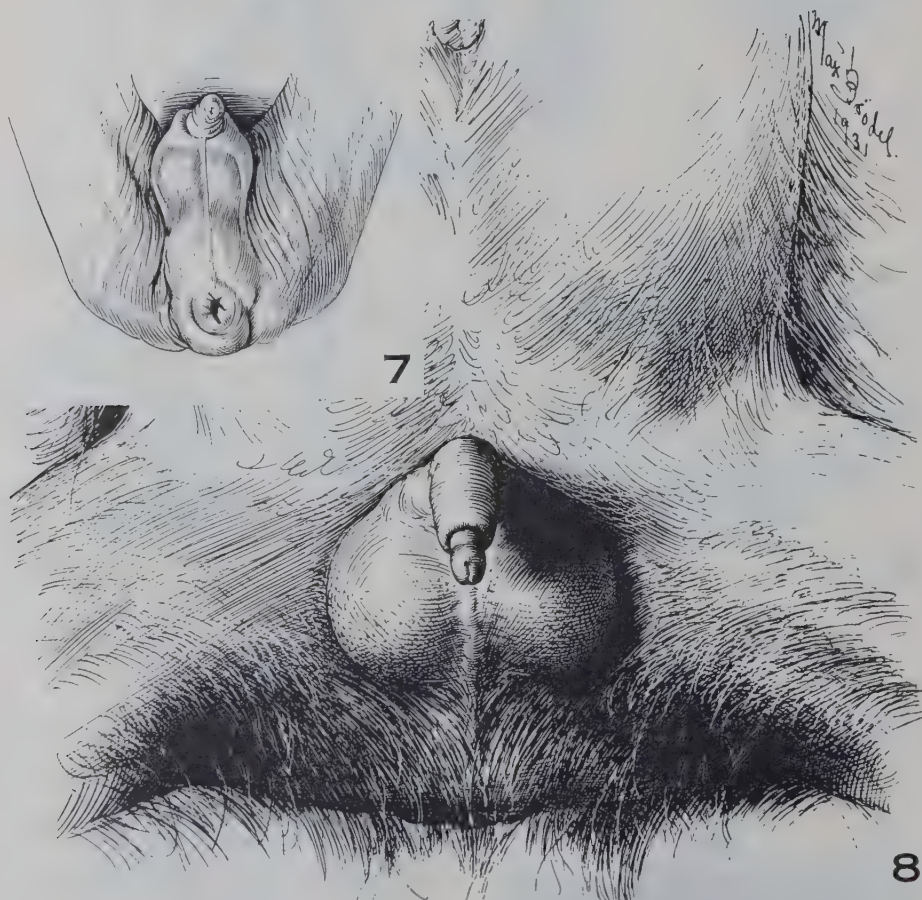
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PLATE 1

EXPLANATION OF FIGURES

- 1 The external genitalia of a male macaque fetus. Sitting height 110 mm., 2½ months of gestation. $\times 1$.
- 2 The external genitalia of a male macaque fetus at end of gestation. Sitting height 185 mm. $\times 1$.
- 3 The external genitalia of an infant male macaque. Sitting height 212 mm. $\times 1$.
- 4 The external genitalia of a young adult male macaque, partially dissected. $\times \frac{1}{2}$.
- 5 Dissection of the external genitalia of a male macaque at birth to show the appearance of the scrotum and the penis and the location of the testes. Sitting height 216 mm. $\times 1$.
- 6 The external genitalia of a newborn female macaque. Sitting height 193 mm. The small insert above shows the rima pudendi with the labia minora spread apart.





- 7 The external genitalia of a male chimpanzee fetus. Sitting height 193 mm. $\times 1$.
8 The external genitalia of a juvenile male chimpanzee. Sitting height 416 mm. $\times 1$.

A CYTOLOGICAL STUDY OF THE ANTERIOR PITUITARY OF THE RAT, WITH SPECIAL REFERENCE TO THE GOLGI APPARATUS AND TO CELL RELATIONSHIP ¹

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FOUR TEXT FIGURES AND TWO HELIOTYPE PLATES (TWENTY-TWO FIGURES)

The anterior pituitary has been an object of histological interest for more than fifty years. This interest has been directed largely toward a study of the distinct cell types, two of which, the chromophobes (non-staining, non-granulated cytoplasm) and the chromophiles (with demonstrable cytoplasmic granules) were described as early as 1884 by Flesch. Schoeneman (1892) further divided the chromophiles into acidophiles and cyanophiles (now more frequently known as basophiles), terms which are self-descriptive. These two granular types together with the non-granular chromophobes remain today the only well-established cell types of the normal gland. Although the original cytological interest in the anterior pituitary was elicited by its unique embryology, the great impetus for its study during the last decade has come from the realization of the gland's important endocrine functions. The present work is the result of renewed demands constantly being made by rapidly accumulating physiological data for a better cytological interpretation of the pituitary phenomena. Reviews of the literature concerning anterior lobe histology can be found in the excellent papers of Kraus ('14) and Bailey ('21) which introduce the reader to a wide literature. The present paper is a more

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complete report of data presented in abstract before the American Association of Anatomists in 1932.

The cytological literature dealing with the hypophysis of the rat is limited, and unfortunately, because of species variations, cytological data from other species cannot be applied directly to the rat. Zacherl ('12), Schleidt ('14), Addison ('17), Nukariya ('26), Schenk ('27), and Lehmann ('28) have discussed the structure of the rat pituitary in connection with castration experiments, and reference will be made to some of their findings.

NORMAL HISTOLOGY

The normal histology is ably reviewed by Nukariya, from whose description certain salient features are briefly restated. He reports in male rats, ranging in age from 5 weeks to 18 months, that the anterior lobe is composed of 54.5 per cent chromophobes, 31.4 per cent acidophiles, and 14.1 per cent basophiles, while in females the percentages are almost identical: chromophobes, 52.9 per cent; acidophiles, 31.6 per cent; and basophiles, 15.5 per cent. These figures agree closely with the cell proportions given by Rasmussen for the human male hypophysis, viz., chromophobes 54.3, acidophiles 34.8, and basophiles 10.9.² The regional distribution of cells is also of interest. The acidophiles are described as most plentiful in the central portions of the gland and surrounding the pars intermedia cleft. The basophiles, on the other hand, are more peripherally located and are especially numerous at the lateral junctions of the pars intermedia with the anterior lobe. The chromophobes are found in groups quite regularly distributed throughout the gland. Our own observations confirm in general this arrangement of cells in the rat.

THE CHROMOPHOBES

The chromophobes are often described from haematoxylin and eosin preparations as those cells which are not acido-

²It should be pointed out, however, that the results of Nukariya, as judged from data given, cannot have anything like the statistical value which data of Rasmussen possess.

philes or basophiles. In certain of the chromophobes the cell boundaries stain with difficulty and, since so little cytoplasm is present, a group of such cells has the appearance of a compact aggregate of nuclei. The whole mass resembles a syncytium. With fixatives such as Regaud or Champy, followed by proper staining, the cell margins become evident. The slightly chromatic nucleus with one or two intensely staining nucleoli is surrounded by a slender margin of cytoplasm. The clear cytoplasm, devoid of granules, except for the few granular mitochondria which are demonstrated by appropriate fixatives, takes only a faint basophilic stain even after the tissue is much overstained. Hence the name chromophobe, which has come into general English usage as a substitute for the later German term, Hauptzellen (chief cells). Chromophobes with increased amounts of cytoplasm are also to be found and these not infrequently show an indistinct granular cytoplasm. Such cells are interpreted as illustrating a phase of the transition between chromophobe and chromophile. See page 166.

The Golgi apparatus is well demonstrated by the Kolatchev (Nassonov) technique, and is found to be either a narrow and elongated network closely applied to one side of the nucleus, or a compact spherical mass lying free in the cytoplasm. These two types agree in morphology with the Golgi of acidophiles and basophiles, respectively, and they will be treated more fully under the section dealing with cell relationships.

THE ACIDOPHILES

With most techniques, the acidophiles, because of their numbers and brilliant staining, constitute the most conspicuous cell type of the anterior pituitary. In the rat the largest acidophiles are cells of about $10\ \mu$ in greatest dimension. They are often round or ovate, but not infrequently angular or elongated, especially when lying grouped along a blood capillary. Such a row of cells is seen in figure 1. The nucleus is central with a prominent acidophilic nucleolus and usually a second nucleolus of basophilic character. The granular cyto-

plasm may appear homogeneous and non-granular after certain fixatives or when heavily stained. The granules are best defined by chrome-osmic or chrome-formic fixatives, which also preserve the granular mitochondria. The mitochondria are, however, masked by the acidophilic granules unless

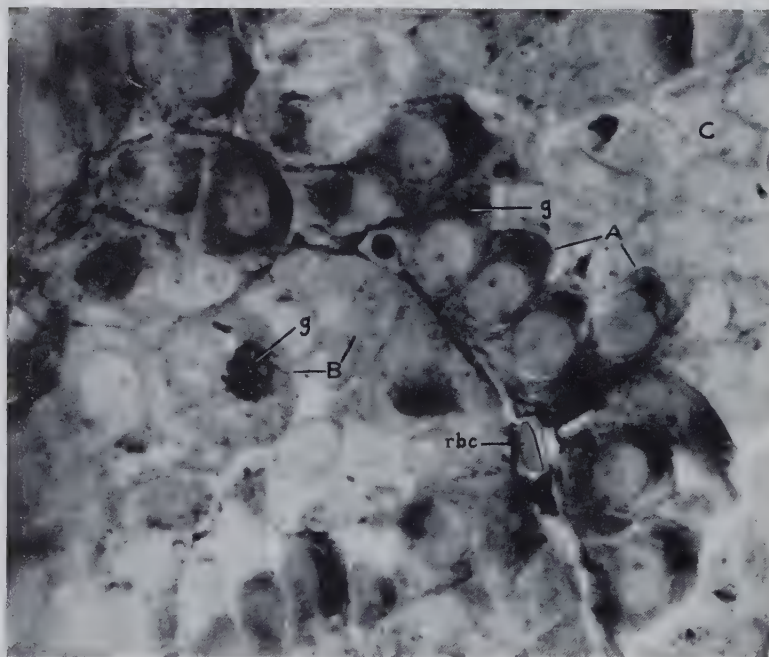


Fig.1 Untouched photomicrograph of cells of the anterior pituitary of an adult male rat. Note alignment of acidophiles along a capillary, with the Golgi polarized away from capillary. A, acidophiles; B, basophiles; C, group of chromophobes; g, Golgi apparatus; rbc, red blood cell. Zeiss 1.5 oil immersion. Nassanov Kolatchev, counterstained preparation.

especially differentiated (Severinghaus, '32). When acid fuchsin staining is properly controlled after Champy-like fixation, the mitochondria remain as large brilliant fuchsin granules among the finer orange-red granules of the cytoplasm. These granular mitochondria, which can be demonstrated by Janus green in fresh smear preparations, are never

abundant and are frequently polarized in the meshwork of the Golgi.

The Golgi apparatus consists of a basket-like network closely applied to one side of the nucleus. Occasionally it extends as a fine branching meshwork throughout a larger portion of the cytoplasm, but it is typically restricted as a cap to one side of the nucleus (fig. 8). In the mouse pituitary Urasov ('28) has stated that in the acidophiles which adjoin a capillary, the Golgi is polarized toward the capillary lumen, and he joins with many others in pointing to this position as indicating that the Golgi is concerned with the discharge of secretory products from the cell to the blood stream. Such a polarization cannot be confirmed in the rat. On the contrary, the Golgi is almost invariably polarized away from the capillary. Figure 1, by chance, shows eleven acidophiles adjoining two capillaries in a single section, and in each cell the nucleus lies between the Golgi and the capillary lumen. The idea that the polarization of the Golgi is an indicator of the portal of secretion is losing significance due to much accumulating experimental evidence that the Golgi does not change its position when the portal of secretion in the cell is changed. This has been shown by Wagschal ('31) in the pituitary-stimulated thyroid of the guinea pig and independently by Severinghaus and Schockaert (unpublished) in carefully controlled studies on the thyroid of the duck and other species, where the portal of excretion of the cells is reversed after pituitary implants without altering the apical position of the Golgi. Comparable results were reported by Uhlenhuth ('32) in a similarly activated thyroid of the California newt, and Okkels ('32) in normal and abnormal human thyroids. None of these findings deny to the Golgi a participation in the elaboration of secretory products, but they are strong evidence against the claim that the position of the Golgi can be considered an indicator of the pole of excretion.

THE BASOPHILES

The basophiles are the largest of the anterior pituitary cells. The largest measure fully $25\ \mu$ in diameter. They are often round in section or polyhedral when compactly associated. The nucleus is always markedly excentric. In general character it differs little from the nuclei previously described in the chromophobes and acidophiles. Its clear ground substance stains palely basophilic. The nuclei of all cells are cytologically typical resting nuclei. Mitoses almost never occur among the pituitary cells. They have been reported on a very few occasions (Nukariya, Schenk) in the basophiles of the female rat 2 or 3 weeks following castration, and I have seen them only once in the rat, a female injected with prolan.

The cytoplasm of the basophilic cells shows a wide variation in structure. It may be either finely granular or have granules of a coarse, irregular or flocculent type. A wide variation also exists in the amount of granulation. This granular structure, in fact, is often entirely absent, and one finds in its place a vacuolation of the cytoplasm, giving the cell an alveolar appearance. These vacuoles are usually smallest near the center of the cell and increase in size as they approach the cell borders (fig. 13). Such alveolar structure is not to be confused with vacuolation in the formation of the so-called castration cells.

Quite obviously these cytoplasmic differences in the basophile are manifestations of changes in the cell to be associated with its secretory function, and the temptation is always great to arrange a cytological series which can be pointed to as illustrative of stages in the elaboration of the secretory product. The writer declines at present to yield to such temptation, because of the conviction that we are still far from certain whether the end secretory products are granules, vacuoles, or lipoidal and hyaline masses, all of which are to be found within the gland. These products at various times have been described as secretory substances, either extracellular or even within the capillaries. None of these de-

scriptions has been convincing, and it would seem best at present to reserve cytological judgment both as concerns the structural manifestation of the final secretory product and the mechanism of its release and transportation. It would seem unjustified, also, to attempt to subdivide the basophiles into types on the basis of their cytoplasmic variation. Such attempts are apt to lead only to confusion, and to complicate an already difficult cytological picture.

The Golgi apparatus of the rat's basophile is a constant cytoplasmic structure, and its definite morphology identifies the cell regardless of cytoplasmic variations. It is somewhat spherical in shape and lies in the cytoplasm, separated from the excentric placed nucleus. Both by its form and its position, it is sharply contrasted with the acidophilic Golgi. In the smaller basophiles the Golgi body appears as a hollow sphere with one or two invaginations. The wall of the sphere osmicates, and there is indication from a study of progressive osmication and from bleached preparations that osmic is deposited on the outer and inner surfaces of the membranous wall leaving a chromophobic Golgi substance between. The Golgi body enlarges with the enlargement of the cell and this expansion frequently results in a separation of the wall into a system of anastomosing strands, giving to it the appearance of a fenestrated membrane. One or more clear vesicles are normally associated with the Golgi body, and in negative image preparations, one gets the impression that the walls of the sphere are canals opening into these vesicles.

The general morphology of the Golgi body is shown in figure 12 which is a drawing of a model made up from a study of a large number of cells. Optical or actual sections through the Golgi give a variety of configurations, some of which are shown in the camera lucida drawings (fig. 2). The negative image of the Golgi body, which usually appears as a clear ring, sometimes enclosing one or two smaller rings, which are sections of the invaginations, most readily reveals, through deep focusing, its spherical character.

The cytoplasm contained within the Golgi region is distinctly different in character from the general cytoplasm of the cell. It is much more compact and finely granular. With the Altmann acid fuchsin, acid violet-methyl green technique, it stains darker and more violet, in contrast to the paler blue of the outer cytoplasm. The violet color is apparently due

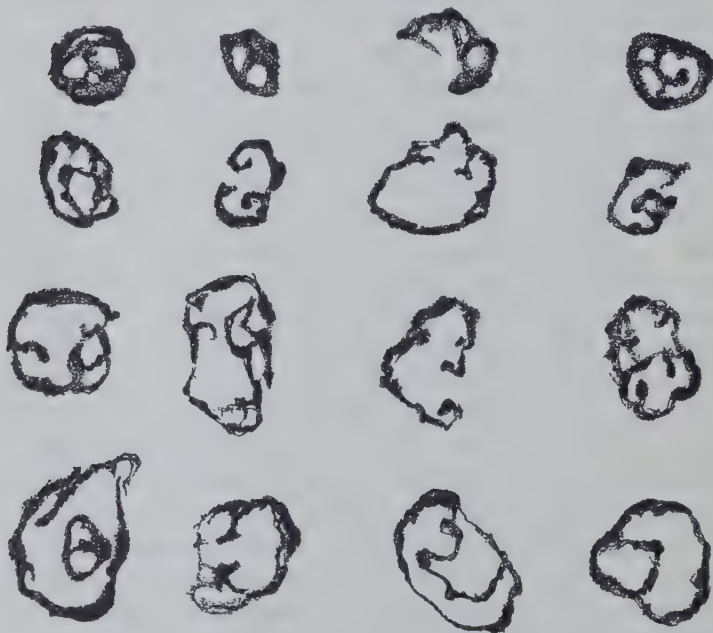


Fig. 2 The Golgi apparatus as seen in sixteen different basophile cells from the anterior pituitary. Compare with the three dimensional drawing, figure 12. Camera lucida drawings.

to the distribution of fine fuchsin granules through the dark dense blue cytoplasm.

It is important to note that this Golgi is present in all basophiles of the normal pituitary as well as in the basophiles and the castration cells of gonadectomized rats. It is identical with the structure known as the macula (Addison, '17). It is more readily demonstrated in the pituitary of the cas-

trate, owing to the increased number of large matured basophilic cells. Its prominence here is interpreted as a correlation to the cells' increased secretory activity. In the fully developed 'castration cell,' as well as in early formation stages of these cells, where several small vacuoles are still present, the Golgi is of this same basophilic type, showing unquestionably that the castration cells are modified basophiles (figs. 22 to 26). The prominence given to the basophilic Golgi region and the somewhat confusing references to it in recent literature invite additional attention to it. Reese and McQueen-Williams, in Evans' laboratory, treat the region of this basophilic Golgi both in their text and in their figures as if it were rarely present in the normal rat. In using it as an indicator of the castration effect upon the hypophysis they are on dangerous ground, for the Golgi (macula) region is a constant feature of every basophilic cell. Figure 5 shows a field of basophiles from the pituitary of the normal male rat. Every cell shows the negative image of the Golgi apparatus. The importance of accurate cytological descriptions becomes evident when structural features of the cell are employed as physiological indicators.

The negative image of the basophilic Golgi, the clear ring, has been clearly pictured by previous investigators, where it has been termed the 'ringbildung' of the cell. Nukariya ('26), Schenk ('27), and Lehmann ('28) all figure it and show also the denser protoplasm within the ring. These figures are, for the most part, of pituitaries of castrates, although Nukariya shows the 'ringbildung' in the basophiles of the normal rat. Schenk (p. 492) obviously considers this 'ringbildung' as an indication that the cell has undergone modification into a castration cell. In describing these cells he says:

In the vicinity of the nucleus one finds in many cells a place which, because of its compact texture, appears as a fairly well defined intracellular structure, similar to a cell sphere. From this structure, in time, the far-reaching changes take their origin. Next a clear area caps this darkened portion and then

a clear intracellular cavity, which contains a plasma-like substance; finally, with a coagulum or else apparently substantially empty, so that the 'Siegelringforms' of Schleidt arise.

Guyer and Claus are also of the opinion that the Golgi apparatus is associated with the formation of the castration cell vacuole. Such a relation cannot, however, be seen in their figure 17, page 237—where the Golgi has no closer relation to the vacuole than does the nucleus.

From my own observations it appears that this 'ringbildung,' which is the Golgi apparatus, takes no part in the formation of the vacuole in the castration cell, but is itself pushed aside with the nucleus as the vacuoles enlarge and coalesce. This is most clearly shown in the Golgi preparations (fig. 23 to 26). In this respect my observations are in direct agreement with those of Nukariya who states that the 'ringbildung' is crowded to the periphery of the cell, undergoing with the nucleus an elongation through compression beneath the cell wall. Even Schenk (p. 493) figures the fully formed signet cell in which both the peripheral nucleus and 'ringbildung' are still present and pushed aside, and it is difficult to reconcile his description with his figure. These observations establish, then, the fact that this 'ringbildung' is the negative image of the basophilic Golgi body, described above, and that it does not play a direct part in the formation of the vacuole of the castration cell. This latter fact is in a way disappointing, for it would be expected that the Golgi, so frequently associated with the elaboration of glandular secretion, could be correlated with the direct formation of these large vacuoles in the castration cells, which, in the rat, have been looked upon as storage for the accumulating sex maturing hormone (Engle, '28).

The mitochondria of the basophilic cells are identical in appearance with those of the other cell types. They appear as brilliant fuchsin granules, which vary in size from minute granules to spheres as large as the nucleoli. The smaller mitochondria are frequently massed about the Golgi region, which often appears as a center from which they radiate into

the cytoplasm. They are much more numerous in the basophiles than in the acidophiles, and they reach maximum size and number in the largest basophiles. However, in basophiles of the castrate, the mitochondria appear to be diminished. If we attribute to these basophiles of the castrate an increased secretory activity, it appears that the mitochondria, which increase with the general growth of the cell, are again decreased during its functional activity. Further than this, no positive correlation has been established between mitochondrial and other cytoplasmic variations. There is no evidence at present, from my studies, to show what part, if any, the mitochondria play in the actual elaboration of the cell's secretion. Urosov has noted this increase of the chondriosomes during growth, and their subsequent decrease during the late secretory phase of the cell. He therefore concludes that the secretion is elaborated at the expense of the chondriosomes under the influence of the Golgi apparatus.

As intimated earlier, it has been impossible to arrange with certainty the cytoplasmic variations, to be found among basophiles, into a series which is illustrative of stages in the cyclic elaboration and discharge of secretion. Until that is possible, any more definite statement concerning the relation of the Golgi or the mitochondria to the elaboration of its secretion must be withheld. Such information is being sought in the rat and in other species by further studies involving experimental physiological changes in the gland. It should be mentioned that the compact, finely granular character of the cytoplasm within the confines of the Golgi body, in contrast to the remaining cytoplasm, might be looked upon as indicating that this region is the site of granular as well as mitochondrial elaboration and growth, and in this respect might be suspected of having a relation to the elaboration of secretory product.

CELL RELATIONSHIPS

The relationship of the chromophobes, acidophiles, and basophiles to each other has been subject to continual dis-

agreement since these cells were first described. In recent years, since the production of several important hormones has been ascribed to the pituitary, the matter of cell relationship has gained a new importance. It is established that cellular changes in the gland accompany certain changes in the potency of the secretion. The increase in the number and size of basophiles in the rat following castration is a case in point. What is the source of these basophiles? Mitoses in the adult gland are rare, even when it is undergoing marked cellular changes in response to a physiological change in the organism. This, and other facts have led many observers to conclude that some interchange between the three types of cells must occur.

Two general theories of cell relationship have long since been advanced, and both are still supported in the current literature. The first assumed that the chromophobes, acidophiles, and basophiles represent three quite distinct types of cells, while the second looks upon these cells as representing various forms or physiological stages of the same cell type. Under each of these theories the actual relationships of the cells are variously regarded. It would seem advisable to review briefly these suggestions in the literature before submitting the evidence which the present study has brought to bear upon this controversial question.

The earliest investigators, including Flesch, Dostojewsky, Lothringer, and Schönemann, regarded the anterior lobe cells as distinct types. St. Remy (1892), however, using Altmann's method, found acidophilic granules in all of the cells, and for this reason looked upon all cells as being various stages of a single type in which the ground cell was the acidophile. Through loss of granules, the acidophile became a chromophobe. Basophilic cells with acidophilic granules were regarded as evidence that interchanges took place between the acidophile and the basophile. As Bailey ('21) points out, St. Remy was unquestionably mistaking the mitochondria of the basophile cell for acidophile granules. Benda ('00), agreeing with St. Remy that the various cells of the anterior

pituitary are functional stages of the same cell, had, however, quite a different idea regarding their relationship. He derived the acidophiles from the chromophobes, and described transitional stages in which small cells, chromophobic in character, showed a gradual growth and an accompanying accumulation of acidophilic granules scattered through their basophilic cytoplasm. Benda further described a complete series of transitional stages whereby the acidophilic cells developed basophilic regions of fine dust-like granules among which fewer and fewer acidophilic granules were to be found until eventually there were formed these large cells which he did not name, but which were certainly the cyanophiles of Schönmann (basophiles). These cells were occasionally vacuolated, one feature which led Benda to suspect the basophile of being a degenerating cell.

Perhaps most prominent among the present advocates of the unitarian point of view is Collin, who, like Benda, believes that the chromophobe is the progenitor of the acidophile. The acidophile being the secretory stage, may discharge its granular cytoplasm and degenerate, or it may, by this cytoplasmic loss, become a chromophobe and again repeat the cycle. Certain acidophiles by transformation become basophiles. A most interesting conception of Collin is his assumption that both acidophiles and basophiles may at times give rise to new chromophobes by an endocytogenetic budding or rebirth. This most unusual cytological phenomenon should certainly be critically reviewed (fig. 3). It is quite probable that Collin's endogenous cells are, in fact, cells which are superimposed. In the monkey (fig. 11), I have frequently found a chromophilic cell cupped about a chromophobe and only a careful study of serial sections reveals that the apparently enclosed cell is actually superimposed. One could not tell from the illustration, which closely simulates that of Collin, the true nature of the relationship of these cells.

Of the earlier investigators who looked upon the different cells of the anterior pituitary as distinct types, Trautmann

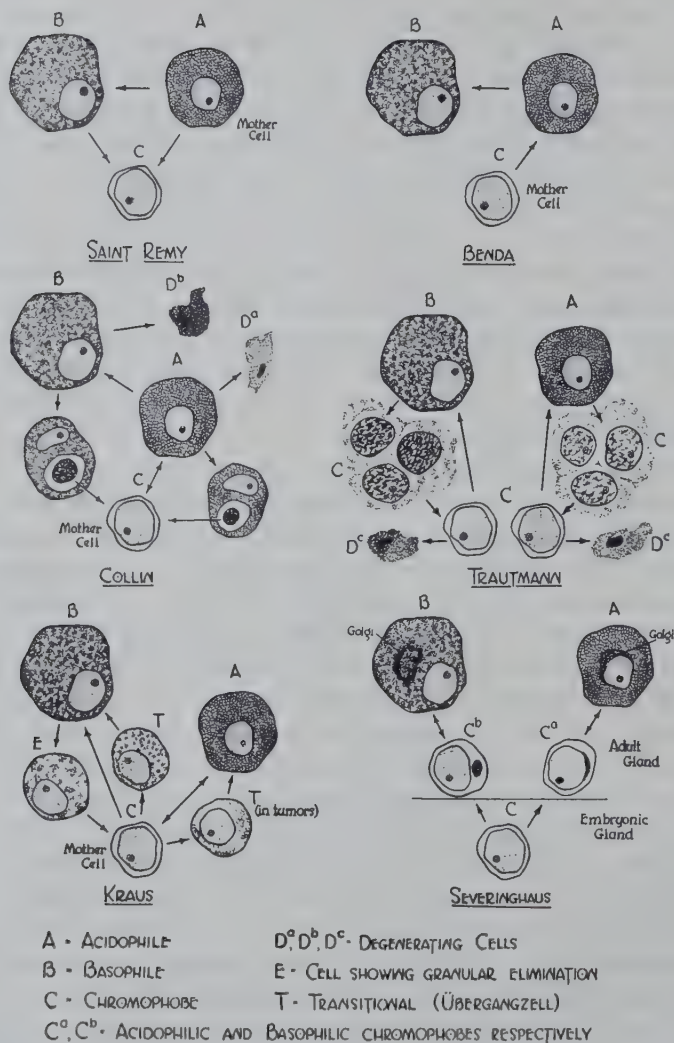


Fig. 3 Diagrams to illustrate various ideas of relationships among the cells of the anterior pituitary.

('09) carried on, perhaps the most extensive studies. From an examination of many species he reached the conclusion that basophiles and eosinophiles were distinct types, and that it was difficult to understand any direct interchange or transition between them. He described the cell cycle for the basophile, showing that by loss of its granules it returned to a small chromophobic type. He assumed that some of these cells degenerated, but most of them again filled with granules to reform basophiles. He believes that the same process occurs in the acidophiles, but is at a loss to understand why so few acidophile-forming chromophobes are encountered as compared to the number of the basophilic line. Trautmann's theory is not so different from that of Erdheim and others. Erdheim ('09), however, pointed out that once a chromophile had lost its granules, it was no longer possible to determine whether it was of an acidophilic or basophilic line.

Kraus ('14) reviews the various theories of relationship and then presents in excellent fashion his conclusions based upon an extensive study of normal pituitaries and some twenty-five adenomas. Kraus follows fairly closely the interpretation of Trautmann but emphasizes a definite 'Übergangszelle' (transitional cell)—an intermediate developmental stage between chromophobe and basophile. These cells differ from the chromophobes by their sharp cell boundaries but they are still without any basophilic granules. Kraus states the cell relationships in the following clear and concise terms:

The chromophile cells arise from the chromophobes, and by the reverse process chromophiles again become chromophobes. Whereas, under normal conditions, the acidophiles appear to arise direct from the chromophobes, in the tumors they often arise through an intermediate cell type, the 'Übergangszelle'. Basophilic cells arise in tumors as well as in the normal hypophysis through the 'Übergangszelle', but also direct from the chromophobes, a fact which is not clearly seen in the normal gland, but probably true. The reversion of both granular types into chromophobes through elimination of granules and loss of cytoplasm occurs also in tumors. It is very questionable if these chromophobes, formed by de-

differentiation of the chromophile cells, can again become chromophiles,—if this cycle of building up and dedifferentiating can take place two or more times for the same cell. This question cannot be answered at present, since we are not able to determine whether these chromophobes, which are in the process of becoming chromophiles, are, if I may use the term, primary chromophobes, cells of the first generation, or whether they have arisen from cells which have already dedifferentiated from the chromophiles.

From these studies, I divide the cells of the human hypophysis on purely histological grounds into granular and non-granular cells. Under the non-granular I distinguish chromophobes and *Übergangszelle* (transitional) cells, and degranulated cells, which represent the final stage in the reversion of chromophiles to chromophobes. The granular cells are the acidophiles, basophiles and pregnancy cells, which are a special variation of the acidophiles. From this point of view I have also classified the adenomas as follows: typical and atypical chromophobic adenomas, transitional, acidophilic, basophilic and pregnancy cell adenomas.

The study of adenomas is of great importance from the standpoint of cell origin, for adenomas are as a rule of pure types. As Kraus pointed out, the presence of these pure tumors is itself strong evidence in favor of regarding the cells of the pituitary as distinct types. This point of view is accepted by Bailey and Rasmussen and is otherwise widely held by investigators in this field, both from cytological and physiological considerations.

What is the origin of the chromophilic adenomas? Do basophilic or the more common acidophilic adenomas result from initial chromophobic hyperplasia, or do these cells actually divide under pathological conditions? Kraus has described tumors in which both chromophobes and matured acidophiles are present. In such cases he considers it merely a matter of time until all the chromophobes will become transformed into acidophiles. From his observations he concludes that the majority of hypophyseal tumors of the granular type have begun as chromophobic tumors. He refuses, however, to rule out the possibility that chromophilic tumors may at times develop directly from chromophilic cells. In this respect

the observations of Cushing are most interesting, for he finds mitotic figures much more frequent in pure acidophilic adenomas than in the chromophobic type. This means that the acidophilic cells, by direct cell division, are responsible for the hyperplasia. It leaves us, moreover, to explain why mitoses should be rare in a tumor composed of chromophobes, the mother cell type where cell division should normally be expected, whereas in the acidophilic tumors, composed of highly differentiated and supposedly undividing cells, cell division is much more frequently observed.

The chromophobic tumor, in contrast to the chromophilic types, is regarded as non-secretory and produces its symptoms only after reaching an advanced stage, at which time, because of its large size, it distorts vision through pressure upon the optic chiasma or is discovered because it interferes mechanically with the secretion of the chromophilic cells of the pituitary by literally crowding them out. Such an adenoma is, therefore, first discovered and operated at an advanced stage. It is conceivable that mitoses may have ceased in such a tumor at this stage. It is even possible that purely physical limitations of space in which readily to expand may act as a check to cell proliferation even if the capacity to divide is still inherent. Chromophilic adenomas, on the other hand, are early recognized and are surgically removed at a much earlier and more active stage in their development. Such tumors might be expected to show the more active cell proliferation. Obviously, these acidophiles acquire the capacity to divide under certain pathological conditions, but even the capacity is limited, as is evidenced by the fact that when portions of an acidophilic adenoma are removed, the remaining cells show little tendency to renew active compensatory hyperplasia (Cushing).

A direct bearing upon the problem of the relationships of the pituitary cells is found in a study of the Golgi apparatus in the anterior lobe of the rat. It was mentioned previously that in the rat, the Golgi apparatus of the basophile has a definite and constant structure entirely distinct from that of

the acidophile. Urosov ('28) differentiated between acidophiles and basophiles by morphological differences in their mitochondria and size differences in their respective Golgi apparatus. Atwell ('28) also referred to a difference in the cat. It has not been possible to demonstrate striking morphological differences between the Golgi of acidophilic and basophilic cells in all species. In the rat, the Golgi differences between these two types of cells are not merely size differences but are differences in fundamental form and in location within the cell. Further study revealed that these two distinct types of Golgi could be found also among the chromophobes.

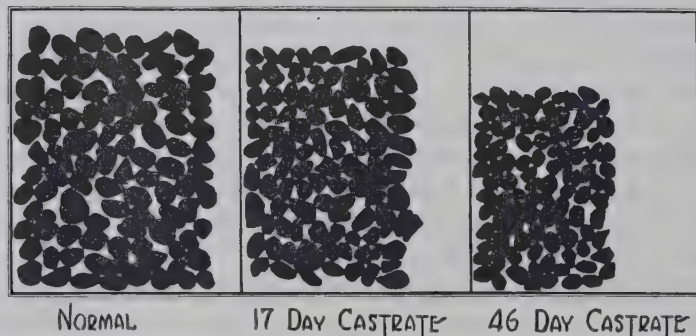
The previously undifferentiated and otherwise indistinguishable chromophobes are, therefore, separated by their Golgi structures into two distinct groups, one acidophilic, the other basophilic in character. If we view the chromophobes as progenitors of the granular cells, we see that already in this early stage, certain cells are destined to become basophiles and other acidophiles. And if we believe, as the writer does, that these chromophiles may revert into chromophobes, then it follows that a basophile dedifferentiates into a basophilic chromophobe, and the acidophile, likewise, into an acidophilic chromophobe. That these may again form their respective granular cells is regarded as highly probable. Thus Bailey's statement that "all the evidence, at present available, therefore indicates that these two types of cells (basophiles and acidophiles), although originating from indistinguishable embryonic cells, develop on constantly diverging lines," must be amended, but it becomes even more firmly grounded through the demonstration that the embryonic cells are themselves differentiated. From a cytological point of view it would seem definitely established in the rat that direct transitions between the basophile and acidophile do not take place, for it is unlikely that a complete change in the morphology of so definite a cytoplasmic component as the Golgi apparatus would occur. Furthermore, the demonstration of a Golgi apparatus in the chromophobe identical in structure with that in a chromophile makes it necessary to associate

these cells in a developmental cycle. These various theories of relationship are diagramed in figure 3.

Evidence that the chromophobes are progenitors of the chromophiles as well as products of chromophilic dedifferentiation is seen also in preliminary studies of the pituitaries of castrated rats. It has been established by repeated researches that the basophiles are greatly increased in number in the anterior lobe of a castrated rat (see p. 150). Several observers reported also an accompanying decrease in the number of acidophiles. If this be true, and if the diphyletic theory of cell relationships, as outlined above, is correct, then in the pituitary of a castrated animal, two simultaneous processes must be taking place. Basophilic chromophobes must be differentiating to increase the number of basophiles, and acidophiles must be reverting to acidophilic chromophobes. These processes together should greatly increase the proportion of acidophilic chromophobes to basophilic chromophobes. In the pituitary of a 46-day castrated male rat, osmicated to give a general Golgi impregnation, one sees this exact cytological picture. The basophiles are greatly increased, so much so that accurate cell counting is not necessary to establish the fact. Acidophiles are apparently decreased although this decrease is not so striking as is the increase of the basophiles. It is a fact easily determined, however, that castration results in a progressive decrease in the size of the acidophilic cells. This is seen in one hundred consecutively drawn acidophilic cells from the normal adult male rat, a 17-day castrate, and a 46-day castrate, respectively, shown in area tracings (fig. 4). Here we would seem to have evidence, in view of the repeatedly reported reduction in eosinophiles, that some of these cells are dedifferentiating into chromophobes. Furthermore, an examination of serial sections of the 46-day castrate pituitary shows that the proportion of acidophilic chromophobes to basophilic chromophobes has strikingly increased. This increase is so obvious that the writer ventures to report it at this time, even though he is committed to the policy that proportional cell changes in the

anterior pituitary can be established only by accurate counting of serial sections in a large number of individuals. Additional observations must be awaited to firmly establish this evidence on cell relationship from the pituitaries of castrates, and it is also hoped that studies now in progress on the foetal pituitary may reveal the stage at which the embryonic chromophobic cells first become differentiated.

A SIZE COMPARISON
OF
100 ACIDOPHILIC CELLS FROM THE ANTERIOR HYPOPHYSIS
OF NORMAL AND CASTRATED
ADULT MALE RATS



CAMERA LUCIDA TRACINGS — MAGNIFICATION AND TECHNIQUE IDENTICAL

Fig. 4 To illustrate the progressive size decrease of the acidophiles which accompanies castration. Measured areas are in the ratios 207:163:112.

SUMMARY

1. The full sized acidophiles of the anterior pituitary of the rat have a cytoplasm filled with granules of even size, which have an affinity for the so-called acid or plasma stains. By proper differentiation of the acid fuchsin stain, granular mitochondria, somewhat larger than the cytoplasmic granules, can be demonstrated scattered through the cytoplasm. The Golgi apparatus appears as a basket meshwork plastered against one side of the nucleus. Clear vacuoles as well as a grouping

of mitochondria are frequently seen associated with the Golgi. The centrally placed nucleus is clear, slightly basophilic and contains one or two large nucleoli. When two nucleoli are present, one is acidophilic, the other basophilic, in character.

2. The basophiles are, at full size, from two to four times the size of the acidophiles. Their cytoplasm may be finely or coarsely granular, flocculently granular (having somewhat the appearance of scattered dust particles), or alveolar, with the vacuoles increasing in size from the center to the periphery of the cell—with no evidence of cytoplasmic granules. The mitochondria are large, spherical bodies throughout the cytoplasm but characteristically massed about the Golgi body as a radiating center. The Golgi apparatus is a spherical body, often doubly invaginated, whose wall impregnates either partially or completely. The cytoplasm within the Golgi body is finely granular and more basophilic. It frequently shows minute granular fragments of mitochondria-like material. The negative image of the Golgi appears as a clear canal enclosing this body of compact basophilic cytoplasm. Depth focusing demonstrates this clear ring to be a section of the wall of a sphere. The nucleus is similar in character to that of the acidophile, but lies strikingly eccentric.

3. This distinct type of Golgi is identical in normal basophiles and in the so-called 'castration cells' showing definitely that castration cells are modified basophiles, a conclusion already quite generally reached from other morphological characters. The presence of this 'ringbildung,' which is the negative Golgi, therefore, cannot be used as an indicator for the castration cell.

4. The Golgi is not polarized toward the lumen of blood capillaries either in acidophiles or basophiles and no evidence can be found to justify using its position in the cell as an indicator of the pole of secretion.

5. The various cytoplasmic aspects of the basophile have not been arranged into a secretory cycle, but it is assumed that the largest cells represent the final secretory phase.

6. The Golgi apparatus increases in size with the general growth of the cell. The largest cells appear to correspond

to stages of highest secretory activity. The Golgi increase is believed to be related to a heightened secretory activity, although the evidence is recognized as debatable.

7. Neither the Golgi apparatus nor the mitochondria can as yet be related with certainty to the elaboration or discharge of specific secretory products in the cells of the anterior pituitary of the rat.

8. The discovery among the heretofore undifferentiated chromophobe cells of two distinct types of Golgi apparatus corresponding to distinct basophilic and acidophilic types, respectively, is strong evidence in support of the theory that the chromophobic cells are progenitors of distinct and divergent chromophilic cell types, and that no transition between the basophiles and acidophiles is possible. Evidence is also presented from a study of castrate pituitaries to indicate that chromophiles revert to their chromophobic form.

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PLATE 1

EXPLANATION OF FIGURES

All remaining figures, excepting 12, are camera lucida drawings.

5 A group of cells from the peripheral region of the anterior lobe of a normal adult male rat. Dark cells in upper left hand corner are acidophiles, in which clear regions, negative image of Golgi, cap the nuclei. Large basophile cells show clear ring (negative image of Golgi) enclosing a denser cytoplasm; eccentric nuclei. Black granular mitochondria present in all cells. Champy fixation.

6 and 7 Two basophilic chromophobes, one with darkly staining nucleus. Golgi apparatus lies above. Compare with basophiles, figures 21 and 17, respectively.

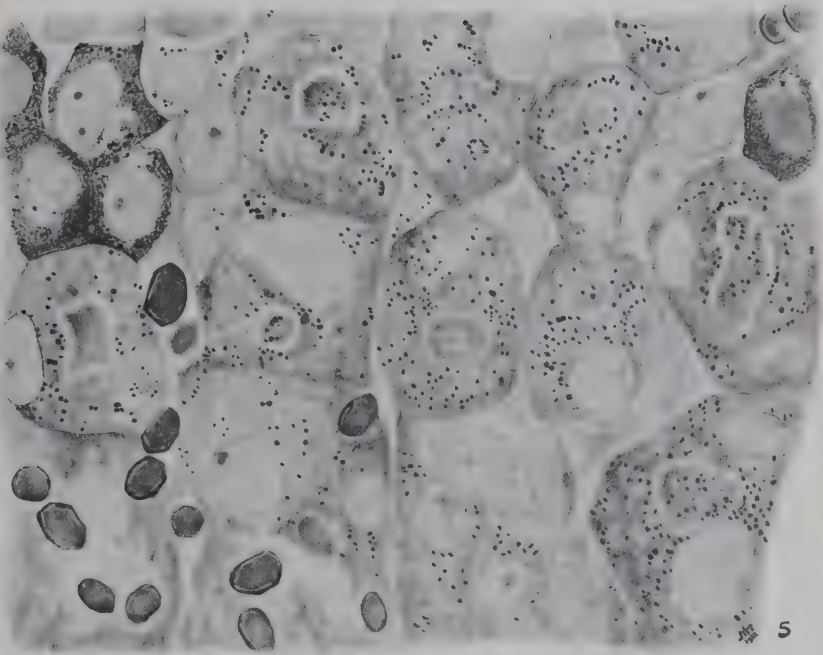
8 A typical acidophile with large even granulated cytoplasm, with granular (black) mitochondria and a Golgi apparatus capping the nucleus.

9 A typical acidophilic chromophobe. Note the scant cytoplasm, the amount of which, however, may vary greatly.

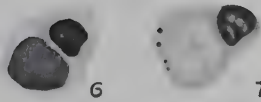
10 Group of thirteen chromophobes. Eight have acidophilic-like Golgi, four basophilic-like Golgi, one acidophilic-like chromophobe has its Golgi in next section.

11 Two chromophilic cells from the anterior lobe of the monkey. Each is cupped about a superimposed chromophobe. Serial sections clearly prove that these chromophobes are not within the cytoplasm of the chromophilic cell, but the similarity to Collins' endogenous cells (fig. 3) is most striking.

12 A drawing of a model of the Golgi of the basophile, showing two invaginations of its membrane-like wall. Often only a single invagination is present. The centrosome appears to occupy the invaginated region.



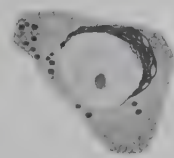
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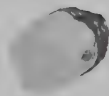
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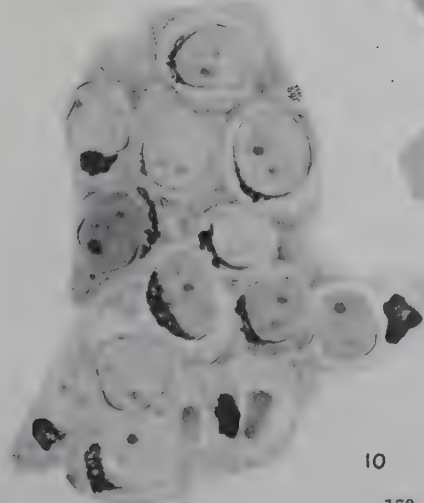
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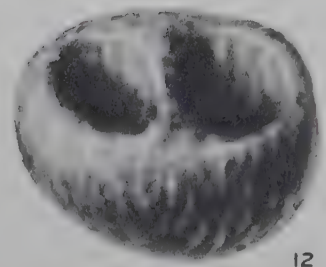
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PLATE 2

EXPLANATION OF FIGURES

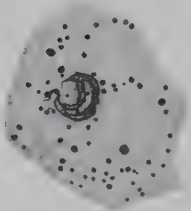
Every cell shows nucleus and the Golgi region. The latter is either osmicated, a negative image, or a dense dark region, depending upon technique used.

13 and 15 Basophiles from anterior lobe of the normal rat. Note alveolar cytoplasm, invaginated Golgi, and numerous scattered granular (black) mitochondria. Nassanov-Kolatchev preparations. Note negative image of Golgi after bleaching in figure 15.

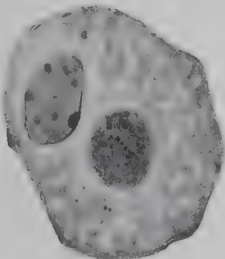
14, 16, 18, 19 Basophiles from the anterior pituitary of a castrated male rat. Note the denser cytoplasm of the Golgi zone, and the varying structure of the general cytoplasm. Figure 20 shows the negative image of the Golgi, a ring within a ring. Mitochondria are not so numerous as in most of the basophiles of the normal rat.

17 and 21 Small basophiles from the pituitary of a normal adult male. Note difference in nuclear staining and compare with basophilic chromophobes in figures 6 and 7.

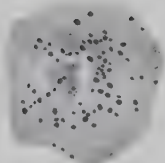
22 to 26 Show castration cells from the anterior pituitary of a 46-day castrated adult male rat. Nucleus and Golgi apparatus are crowded aside by the formation of the vacuole. Figure 25 shows a developmental stage in which four vacuoles of varying size are present. These will later unite. Note that the Golgi is not related to any of these vacuoles.



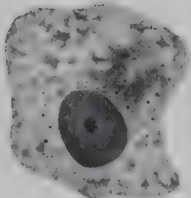
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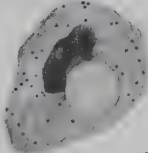
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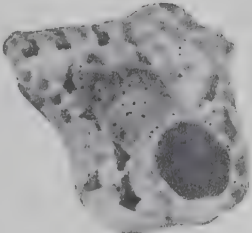
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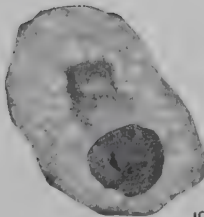
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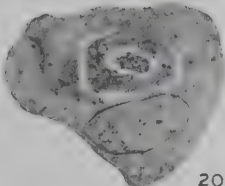
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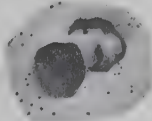
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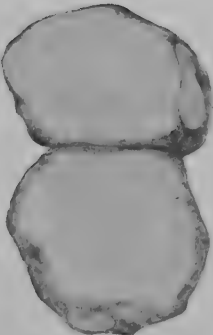
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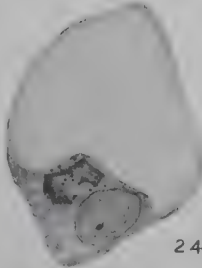
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THE EFFECT OF CASTRATION UPON THE SEX-STIMULATING POTENCY AND THE STRUCTURE OF THE ANTERIOR PITUITARY IN RABBITS ¹

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ONE PLATE (TWO FIGURES)

The anterior lobe of the hypophysis of the rat is known to increase in gonad stimulating power following castration when assayed by direct implantation into suitable test animals (Engle, '29). The increase in potency of this gland in castrated guinea pigs has likewise been definitely proven (Severinghaus, '32). The criterion in both cases for increased potency was the larger ovaries produced by the implantation of the pituitaries of the castrate donors into sexually immature female mice or rats. Wolfe ('32) has confirmed this castration effect on the A.P. gland of the rat by comparing the minimal ovulating doses of the pituitaries of normal and castrated rats when injected in rabbits. In contrast to the results obtained in the rat, he failed to observe any increase in the potency of the female rabbit with castration, when using the same test method. This discordant finding in a pituitary-gonadal relationship of such a fundamental nature seemed to warrant a re-investigation of this problem in the rabbit to determine if its A.P. does not increase in potency as revealed by the ovulation tests in rabbits and also by the implant test in immature rodents.

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Associated with the increased physiological activity of the castrate's hypophysis, an enlargement of the gland with definite morphological alterations of cell types has been reported in certain species. In the rat the anterior lobe hypertrophies with an increase in the number and size of basophile cells and the appearance of the so-called castration cell. For a summary of the work on various species, see 'Sex and Internal Secretions,' chapter 17. In the rabbit, there are conflicting data both as regards the size increase and the cytological changes of the hypophysis following castration. In the light of these conflicting data and of some preliminary work in this laboratory on the cytology of the castrate rabbit A.P., in which an increase in basophiles was found, it seemed all the more worth while to also investigate the problem cytologically.

INCREASED GONAD-STIMULATING ACTIVITY OF THE CASTRATE
RABBIT HYPOPHYSIS AS TESTED ON IMMATURE
FEMALE MICE

Anterior lobes from normal and castrated rabbits were weighed, cut into small fragments with scissors, and implanted by the method of Smith ('27) into immature female mice. Adult rabbits of both sexes were used. The period of castration varied as indicated in the table. The pituitaries of normal females were selected from animals in heat.

Since it was observed that an hypertrophy of the anterior lobe of the pituitary occurred with castration (in the female), equal-sized pieces of the anterior lobes from the two types of donors were used. By weighing the amount of tissue administered, a fairer comparison could be made than by implanting the whole lobe. A rapid weighing balance, accurate to the fourth decimal place, was employed, which allowed the tissue to be weighed prior to implantation without danger of injury due to exposure to the air. Pieces large enough to give a maximum effect were not implanted, for that would have made it difficult to get a differential effect when the physiological limit of response of the immature ovary was approached.

The recipient female mice were litter mates, 22 days old at the time of implantation and were very uniform in weight. Litters of at least three were used so that one uninjected control could be kept in each series. Four days after implantation, the mice were killed and the ovarian weights taken as a criterion of the gonad-stimulating power of the hypophysis.

Table 1 shows the actual amount of tissue implanted into each mouse and the resultant ovarian weights. Taking the untreated control as 100, the relative weights of the ovaries stimulated by normal female A.P. are 182 to 248, with an average of 207, and by castrate female A.P. are 312 to 547, with an average of 462. Thus after implantation with castrate female rabbit A.P. the ovaries of recipients are double the weight of those resulting from implants of normal A.P.³ In the males, the increase in potency due to castration is not so marked. Again taking the untreated control as 100, the ovarian weights stimulated by normal male A.P. are 358 to 426, averaging 393; by the castrate male A.P. they are 433 to 650, with an average of 535. This is about a 36 per cent increase in size of the ovaries. Since the amount of tissue implanted was quite constant, the greater ovarian effect with implantation of pituitaries from castrate rabbits must be due to a higher sex hormone content per unit amount of tissue. This could not have been demonstrated had entire glands been implanted, because the increased effect might have been attributed to the hypertrophy of the A.P. that follows castration, at least in the female.

The data also show a sex difference in the potency of the rabbit pituitary as has been previously reported in rats (Evans and Simpson, '29). With the ovaries of the controls as 100, the average size of the ovaries of the recipients receiving implants of normal female pituitary tissue was 207, as contrasted to 393 for normal males.

³ The increase in the potency of the pituitary was actually more than doubled, for, except with very low dosages, doubling the amount of implanted tissue, and therefore of the administered hormone, does not double the ovarian weights. (From unpublished data of H. M. Clark.)

TABLE 1

Table showing weights of ovaries of immature female mice receiving implants of anterior lobes from normal and castrated rabbits of both sexes

DONORS (RABBITS)			RECIPIENTS (MICE)			PER CENT INCREASE OVER NORMAL IMPLANT	RELATIVE PER CENT WITH CONTROLS AS 100
Type	Weight (lb. oz.)	Days castrated	Weight of implant	Weight	Ovaries		
Normal ♀	5: 13		0.0105		0.0090		187
Castrate ♀	5: 11	81	0.0115		0.0150 0.0045	66	312 100
Normal ♀	5: 4		0.0132	11.3	0.0075		226
Castrate ♀	5: 5	84	0.0133	11.7 11.1	0.0143 0.0034	91	426 100
Normal ♀	5: 4		0.0137	11.5	0.0067		248
Castrate ♀	5: 7	84	0.0135	11.5 10.4	0.0144 0.0027	115	533 100
Normal ♀	5: 9		0.0095	12.4	0.0065		186
Castrate ♀	5: 14	92	0.0103	12.4	0.0161	148	474
Normal ♀	5: 9		0.0099	11.7	0.0062		182
Castrate ♀	5: 14	92	0.0105	12.4 12.6	0.0186 0.0034	200	547 100
Normal ♂	6: 0		0.0095	12.4	0.0170		422
Castrate ♂	6: 6	33	0.0112	11.5	0.0210	24	522
Normal ♂	6: 0		0.0085	10.8	0.0155		390
Castrate ♂	6: 6	33	0.0114	10.1 8.7	0.0260 0.0040	67	650 100
Normal ♂	3: 1		0.0106	12.6	0.0160		390
Castrate ♂	2: 9	78	0.0101	12.1 12.0	0.0205 0.0041	28	500 100
Normal ♂	3: 1		0.0104	12.0	0.0132		426
Castrate ♂	2: 9	78	0.0105	13.7	0.0182	38	587
Castrate ♂	1: 8	34	0.0105	13.0 12.4	0.0192 0.0031	45	619 100
Normal ♂	7: 0		0.0097	12.6	0.0172		358
Castrate ♂	5: 9	100	0.0092	11.8 10.1	0.0188 0.0040	9	470 100
Normal ♂	7: 0		0.0093	11.0	0.0146		406
Castrate ♂	5: 9	100	0.0085	10.7 10.8	0.0168 0.0036	14	466 100
Normal ♂	6: 9		0.0095	10.4	0.0130		361
Castrate ♂	6: 0	123	0.0091	10.2	0.0156	20	433
Castrate ♂	6: 0	123	0.0097	11.6 10.8	0.0168 0.0036	29	466 100

Castration also increases the potency of the pituitary in the female more than it does in the male, as is also the case in rats. Thus with the control ovaries as 100, the castration increased the average response from 207 to 462 in the female, and from 393 to 535 in the male. From our data it is also apparent that the potency of the pituitary of castrate females is equal to or slightly greater than that of the normal male—462 as against 393—but does not equal, in the periods of castration of these experiments, that of the castrate male.

The stimulated ovaries of the recipients showed identical morphological features regardless of the source of the pituitary tissue implanted. In all cases the increase in size was due to follicular stimulation. The uterine weights of the recipients varied widely, so that no significant correlation between the material implanted and the uterine growth could be made. The increased capacity of the castrate rabbit A.P. by this test method is quite in accord with the findings secured on ovulation reported in the next section.

OVULATING CAPACITY OF THE NORMAL AND CASTRATE FEMALE RABBIT A.P.

Ovulation in the rabbit is a delicate test in that it is an 'all-or-none' reaction. Great care must be taken to keep the variables at a minimum. The preparation of the material, its injection, and the condition of the recipient were carefully controlled.

In a series of preliminary experiments using a single anterior-pituitary gland from a normal rabbit, we attempted to make a crude extract after the method described by Wolfe. The anterior lobe was weighed, ground up with about 1 gm. of sterile sand, physiological saline added and then centrifuged, and the solution injected intravenously. The injection volume was kept quite constant, 1 to 2 cc. With doses of 1 to 5 mg. equivalent of fresh pituitary, amounts covering the range which Wolfe found sufficient to give positive ovulation, we were able to secure only an occasional ovulation with the highest dosage with glands from either normals or

castrates. We then made an alkaline extract of fresh glands, but had no higher incident of ovulation with dosages up to 5 mg. In all, seventeen tests were tried before abandoning these procedures. A possible reason for the failure of these methods may be the high loss of potency through absorption when a single gland is extracted at a time.

The method finally adopted consisted in removing the anterior lobes from several animals, weighing them, and dropping them into a large volume of acetone. After several days they were removed and dried thoroughly in a desiccator. They were then weighed again, ground up in a 50-cc. serum tube with a small volume of approximately N/100 NaOH in which they were allowed to remain in the cold for 12 hours. The glands became almost completely dissolved in the alkaline solution. A few drops of phenolphthalein were added and the extract neutralized with dilute acetic acid until faintly pink. The mixture was centrifuged and the residue extracted twice more with small portions of distilled water and once with dilute acetic acid (just sufficient to coagulate the undissolved protein). The extracts were added together and diluted so that 1 cc. of solution equalled 2 mg. of dried rabbit pituitary. The extract was perfectly clear and slightly pink. All injections of any extract were made within a week so as to avoid a possible deterioration. The injection volume was constant in all cases, equalling 1 cc. administered in a single intravenous injection.

Normal pituitary tissue for injection was secured from adult female rabbits in heat. For pituitary tissue of castrates, ten females were used which had been castrated 5 months previously.

For test (recipient) animals, adult female rabbits which showed pronounced signs of heat were used. Past experience has shown that an adult female rabbit in good health (weighing between 2.5 and 3.5 kg.) which has been isolated for at least 20 days and which shows an exceedingly swollen and purple vulva may be considered to be in heat. In spite of this, it is recognized that there will be some difference in

the responsiveness of animals, but the ovaries may be examined by a laparotomy when reading the test and the condition of the ovary will supply additional information on the state that the ovaries were in at the time of the injection. If well-formed corpora lutea are present, the experiment can be discarded. Fortunately, in the sixty rabbits used in these tests,

TABLE 2

Ovulation with A.P. of normal female rabbits (Series I—five donors, Series II—four donors)

NUMBER OF RABBITS	DOSAGE (DRY WEIGHT)	NUMBER OF POSITIVE OVULATIONS	NUMBER OF NEGATIVE OVULATIONS
Series I			
	<i>Mg.</i>		
1	2.0	1 +	0
4	1.5	4 +	0
6	1.0	2 +	4 —
Series II			
1	2.0	0	1
6	1.5	3 +	3 —
6	1.0	1 +	5 —

TABLE 3

Ovulation with A.P. of 5-month castrated female rabbits. (Extract made from glands of five rabbits)

NUMBER OF RABBITS	DOSAGE	NUMBER OF POSITIVE OVULATIONS	NUMBER OF NEGATIVE OVULATIONS
	<i>Mg.</i>		
1	2.0	1 +	0
2	1.5	2 +	0
15	1.0	12 +	3 —

no such conditions were encountered. (Recipients may be used repeatedly if they again come in 'heat,' but a period of 20 days or more must elapse between tests.) An examination of both ovaries was made 15 to 20 hours after the injection for rupture points.

The results in tables 2 and 3 show that castration increased the potency of the female rabbit A.P. as tested by ovulation.

Combining the two series of normal females which had received the 1-mg. dose, the results show three positive and nine negative cases, whereas with the hypophyses of the castrates, the results show the reverse, twelve positives and three negatives. While the number of animals is not large, the uniform results denote an increased capacity of the A.P. of castrate female rabbits to induce ovulation.

THE CYTOLOGY OF THE NORMAL AND CASTRATED ADULT VIRGIN
FEMALE RABBIT

It now seems well established that the anterior pituitary undergoes considerable change due to castration in both the male and the female. These changes are in the main an increase in size, in weight, and in vascularity, as well as a readjustment in the relative numbers of cells of the given types, i.e., the acidophiles, basophiles, and chromophobes. A tabulation of the more important data, covering a large number of the animals available for laboratory study, may be found in 'Sex and Internal Secretions,' 1932, chapter 17, page 808. It is there pointed out that the changes are apparently not similar in all species. Moreover, it can be seen, from the same data, that in the case of the rabbit, with which this paper is concerned, there is much disagreement in the descriptions of the anterior pituitary following castration. For this reason a brief review of the principal literature is advisable.

Fichera ('04), who first studied these castration changes in a variety of species, states that the rabbit pituitary increases in size after castration. He attributes this hypertrophy to a marked increase in the number of acidophiles which he states is clearly evident.

Cimoroni ('08), working in the same laboratory, extended Fichera's observation to a larger series of young rabbits, which were sacrificed approximately 1 month after castration. He confirmed the increase in the number of acidophiles, hypertrophy of the gland, and the marked vascular increase which Fichera had reported.

Kolde ('12) reported his studies on the anterior pituitaries of a series of twenty-one rabbits. This series included normal and castrated young males, young virgin females, as well as multiparous females and older males. He states that in the normal rabbit, both in the male and the female, the acidophiles occupy the greater portion of the anterior lobe, and they are often found grouped into long massive columns. Basophiles are scarce, decidedly fewer in number than either the acidophiles or the chromophobes. He makes the interesting observation that the chromophobes are unquestionably increased in number, occupy the central portion of the lobe, and seem at times to exceed the acidophiles in number. Castration, he stated, always resulted in an hypertrophy of the anterior pituitary (he failed to find it in the one male of his castrate series) and was due to a great increase in acidophiles. Chromophobes and basophiles, on the other hand, decreased in number. In older multiparous females he found that the castration effects seemed less marked, perhaps because the chromophobes, which increase during pregnancy, and the pregnancy cells have a tendency to remain. In some of these cases, therefore, he points out that it is not easy to demonstrate that there are more acidophiles than chromophobes.

Okintschitz ('14) disagrees with previous investigators and states that in the normal rabbit the acidophiles are present in greater numbers than the basophiles and that the chromophobes are still fewer. In the castrate the cell types become, in order of their prominence, chromophobes, basophiles, and acidophiles.

More recently Schenk ('27), after studying a series of seven castrate rabbits, states that in spite of painstaking research he is unable to confirm the often reported increase of acidophiles after castration.

A résumé of this literature shows that whereas some investigators have reported an increase in acidophiles, some an increase in chromophobes, and others no significant changes whatsoever, no one has reported an increase in basophiles.

The data obtained from wet and dry weights of the pituitaries strongly suggest that castration usually, though not invariably, causes an enlargement of this gland (table 4). The hypertrophy is more definite in the female. There seems also to be a slightly higher water content in the hypophyses of the five castrate female animals, but this may not be significant. The potency of the pituitary is increased per unit weight of gland by castration, and this, with the hypertrophy of the A.P., results in an even greater total increase.

Anterior pituitaries of three castrate female rabbits have been examined in this laboratory and found to have a noticeable increase in basophiles. No cell counts were made, but serial sections were studied. The differences between the

TABLE 4
Table giving wet and dry weights of the anterior pituitary used in the ovulation tests

TYPE	NUMBER RABBITS	TOTAL WEIGHT OF RABBITS	WET WEIGHT OF A.P.	DRY WEIGHT OF A.P.	PER CENT SOLID MATTER
		<i>Kg.</i>	<i>Gm.</i>	<i>Gm.</i>	
Normal ♀	5	14.86	0.1130	0.0222	19.64
Normal ♀	4	12.93	0.0992	0.0190	19.15
Castrate ♀	5	14.3	0.1530	0.0270	17.64

castrate and normal pituitary were of such magnitude that either could be identified by a glance in any region of the gland. In one experiment, the cytological and implant data have furthermore been closely correlated. The anterior lobe of the pituitary was removed from a normal young female rabbit, and from a female of similar age and size which had been castrated for 81 days (see table 1, first experiment). One-half of each gland was implanted and the remaining half preserved for cytological study. Fixation and staining followed the procedure previously described (Severinghaus, '32) for the pituitary. The increase in the size and number of basophiles is the striking change in the castrate's pituitary. Figure 1 shows a wide field from the normal pituitary selected because the relative number of acidophiles is minimum. Fields could have been selected to show a much larger num-

ber of acidophiles. Figure 2 shows a field of similar extent from the castrate's pituitary also selected to show the minimum number of acidophiles. The basophiles not only occupy practically the entire field, but such acidophiles as are present have lost their capacity to stain brilliantly. This is true in spite of the fact that the fuchsin staining was forced, as can be seen from the brilliance of the capillary components and the mitochondria. No cells similar to the castration cell of the rat have been found.

The castrate's pituitary showed an uneven distribution of cells. In the central portion, the gland was made up almost completely of basophiles. Peripherally, the relative number of acidophiles was greater. Frequently they appeared in crowded groups, the cells of which were much reduced in size and consisted of a narrow fringe of brilliant acidophilic, granular cytoplasm surrounding a typical nucleus.

The concentration of basophiles in the central region of the castrate's gland, suggests that we have here a similarity to the triangular region described by Rogowitsch as being free from acidophiles. Kolde found this area lacking in acidophiles during pregnancy, and Okintschitz reported it largely composed of chromophobes in the castrate. With Kolde and others we have been unable to establish this region definitely in the anterior lobe of the normal virgin female, although the acidophiles appear to be more numerous peripherally.

The anterior lobe of the castrate pituitary is more vascular than the normal, an observation upon which there is now almost complete agreement (fig. 2). This may account for the increased fluid content shown by dehydration weights reported above.

It is of interest to call attention again to the fact that when the remaining half of the castrate's pituitary, whose cytology has been described above, was implanted into an immature mouse, its sex stimulating potency was much in excess of that of the normal control. Thus we are able to state from the combined cytological and implantation study of this particular gland that an increase in its sex stimulating potency is

directly correlated with a marked increase of its basophile cells.

A portion of each of the other anterior pituitaries studied structurally was not implanted. However, they all showed the same structural changes after castration as did the one discussed above. The functional tests also show a constant increase in potency, an increase which is shown in its greatest magnitude by implantation. We therefore feel convinced that there is a uniform increase in the basophiles after castration and an accompanying increase in the pituitary gonadotrophic hormone. These findings agree with those obtained in rats.

A final word may be expected to explain the variance of these findings from those of previous investigators. It is difficult to understand the reported increase in acidophiles reported both by Fichera and Cimoroni, unless they failed to examine anything more than peripheral regions of the gland. Acidophiles should be demonstrable with any of the histological techniques. The haematoxylin-eosin techniques used by Okintschitz and Schenk are not adequate for accurate separation of basophiles from the chromophobes and it is quite probable that Okintschitz mistook basophiles for the large chromophobes which he reported to be increased. This suggestion is strengthened by the fact that in normal rabbits he states that basophiles outnumber the chromophobes. Attention should also be called again to the fact that these castration changes may be most marked in young virgin females such as we have studied, and that in older multiparous females, as Kolde suggested, a large number of chromophobes and pregnancy cells, being specialized, do not respond as readily to the castrate changes. It is possible, too, that in his animals, Kolde was actually observing the relative increase in basophiles due to castration, and that he interpreted these basophiles as persistent chromophobes or pregnancy cells.

DISCUSSION

From the work on the pituitary-gonad relationship, work which has been done largely on rats and mice, the following fundamental relationships seem established: *a*) the administration of anterior pituitary as first shown by implantation (Smith, '26; Smith and Engle, '27; Zondek and Aschheim, '26, '27) causes follicular growth leading to precocious maturity of the reproductive system; *b*) removal of the gonads induces an increase in the anterior pituitary content of gonad-stimulating hormone, as first shown by Engle ('29); *c*) conversely, administration of oestrin causes a lowering in the gonad-stimulating potency of the anterior pituitary (Meyer et al., '30). The gonad-stimulating effect of the pituitary has now been extended to many forms. The increase in the potency of the pituitary has been shown to take place in guinea pigs (Severinghaus, '32) as well as in the rat, but has been denied for the one commonly used form which does not ovulate spontaneously—the rabbit (Wolfe, '32). Tests on the decrease in the potency of the pituitary with administration of the gonad-sex hormones have not been extended beyond the rat, but the original and fundamental work of R. K. Meyer et al. has been repeatedly confirmed for that form.

That the rabbit is an exception to these apparently fundamental relationships, as reported by Wolfe, in that castration does not cause an increase in the potency of its pituitary has not been confirmed by our work. From the data presented in our paper it is evident that the anterior hypophysis of the rabbit does increase in its gonad-stimulating capacity after castration in both sexes. This increase has been clearly shown in both sexes by the implantation method in immature female mice and has also been demonstrated in the female by the rabbit-ovulation test. This increase has further been shown by quantitative methods to be per unit of tissue.

It must be admitted that our data on hypertrophy of the pituitary after castration are not extensive. Nevertheless, they are definite enough to be strongly suggestive and are in harmony with the more extensive data of Livingston ('16).

We found as did he that hypertrophy in the male after castration takes place but slightly if at all. In the female his data, as well as ours, show a rather uniform enlargement.

We have called attention to the fact that the increase in potency of the A.P. in the female rabbit after castration is likewise greater than it is in the male, as has been also reported in the rat (Evans and Simpson, '29). The greater enlargement of the pituitary in the female after castration thus parallels the increase in potency. Since our data were secured with units of tissue, it follows that the increase in potency per gland in the female is even greater than that shown by our data which are based on units of tissue.

Cytologically, we have also found (in the female, at least) that castration produces definite changes in the A.P. of the rabbit. There is a definite increase in the basophiles. Thus, both functionally and structurally, our findings in the rabbit are harmonious with those reported in the rat. It may be advisable to point out that nulliparous females have been used as donors. It is possible that we might not have secured so great an increase in potency or basophilia had multiparous females been used.

Since two methods of testing for an increase in potency of the pituitary following castration have been employed, it has given an opportunity to evaluate carefully their sensitivity. Of the two, the implant method has shown in a more pronounced fashion the difference between the potency of the A.P. of normal and castrates than has the ovulation test. Indeed, we are inclined to believe, although we have not tried it experimentally, that the ovulation test might fail to show an increase in potency after castration in the male rabbit, for the rise in potency of the A.P. after castration is less in the male than in the female and differences in the gonad-stimulating content of pituitary tissue seem more difficult to demonstrate by the ovulation test in rabbit than by the implant method with standardized immature female mice.

From our data from implantation of the anterior lobes of normal and castrate rabbits a further finding has been se-

cured which is in harmony with earlier work in rats, namely, that the pituitary content of gonad-stimulating hormone is less in the female than in the male. Implants of A.P. from males give ovaries approximately twice as heavy as do the same amounts of tissue from females. We have already mentioned that the increase in potency of the A.P. in males is less than in females. This finding is also in harmony with work on rats, although, within the limits of time of these experiments, the A.P. of the castrate female has not become as potent as that of the castrate male. It seems not unlikely that if the influence of the gonads was withdrawn very early in life, there would be no difference in the potency of the pituitaries between the castrated males and females.

Our findings in this study, although contrary in the main to those of Wolfe, nevertheless have been quite in harmony with those of a similar nature in rats. This seems of interest, for we have been using a form in which ovulation takes place only after mating, whereas in the rat, ovulation is independent of sexual stimulation. Species differences, which are so often confusing in studies on the physiology and structure of the pituitary, seem not to play a part here in the fundamental responses of the pituitary to castration in these two forms which have such pronounced differences in their reproductive physiology.

SUMMARY

1. Castration of the male and female rabbit causes the anterior lobe of the pituitary to increase in gonad-stimulating capacity as tested by implants in immature female mice. The increase in potency is per unit weight of gland. The increase in castrated females has also been shown by the ovulation test in rabbits, a result not in harmony with that reported by Wolfe ('32). Tests by the ovulation method were not made with castrated males.

2. Castration causes an hypertrophy of the anterior hypophysis of the adult nulliparous female rabbit associated with a strikingly increased basophilia.

The anterior hypophysis of the normal male rabbit has a greater potency as tested by quantitative implantation of units of tissue in immature female mice than has that of the nulliparous normal female. Castration (3 months) increases the potency of the A.P. in the nulliparous female approximately 100 per cent and in the male about 36 per cent. Three months after castration the potency of the female A.P. slightly exceeds that of the normal male but definitely fails to attain that of the male which has been castrated for the same period of time.

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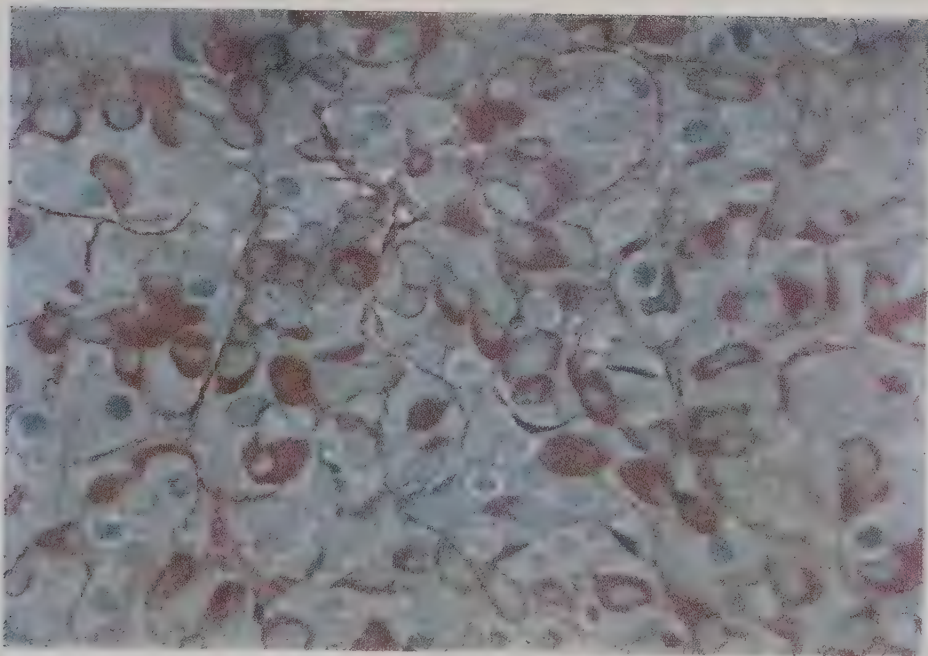
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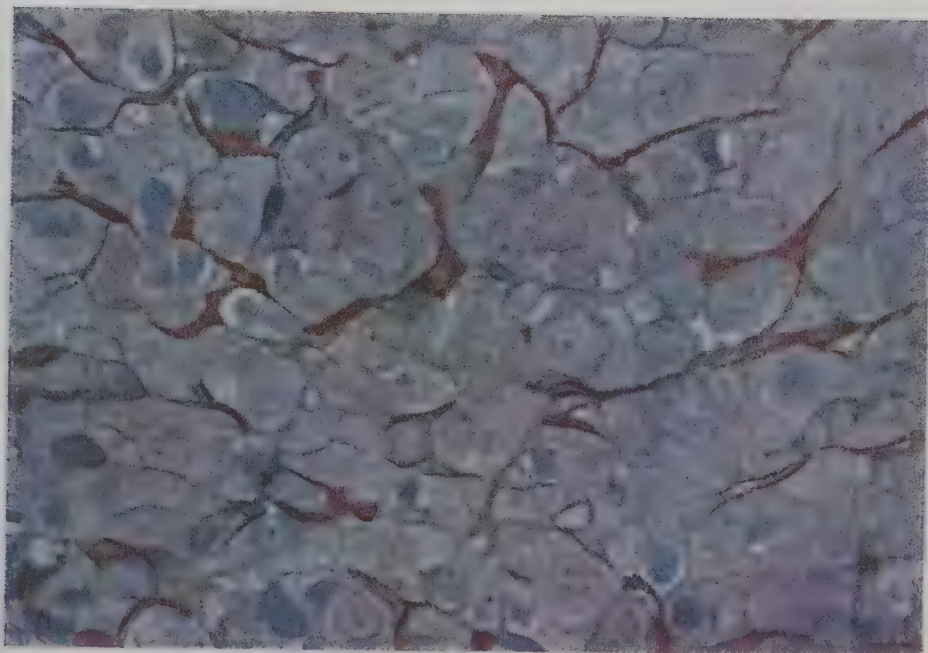
PLATE 1

EXPLANATION OF FIGURES

- 1 Anterior pituitary of a nulliparous adult female rabbit. 850 $\times$.
- 2 Anterior pituitary of a nulliparous adult female 3 months after castration.
Note that basophiles make up almost the entire field. 850 $\times$.
Autochromes not retouched.



1



2

A SEX DIFFERENCE IN PITUITARY SIZE AND INTESTINAL LENGTH IN DOVES AND PIGEONS

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It is known that the hypophysis as a whole, and particularly its anterior lobe, is larger in females than in males of rabbits, rats, cattle and man; in the hibernating woodchuck (*Marmota monax*) it appears that the reverse is true (Rasmussen, '28, '33). McCarrison ('19) weighed a total of sixty-nine pituitaries of pigeons (*Columba domestica*) in normal and in dietary deficient states, and concluded that the female pituitaries are heavier in this species. The present study fully confirms McCarrison on this point and further shows that in ring doves (*Streptopelia risoria*) also a wholly similar sex difference in pituitary size is found. For other species the available data seem too meager to permit any conclusion on the existence or non-existence of a sex difference in pituitary size.

Clinical observations have indicated that hypersecretion of the pars anterior is associated with splanchnomegaly, and at least one case in which the length of the small intestine was doubled has been reported. The present study, based upon suitably inbred material, shows that intestinal length is greater in females than in males in both ring doves and common pigeons. Since earlier study (Riddle, '28) on one of these two species (*St. risoria*) has shown that the average absolute size of the liver and spleen is greater in females than in males, it now appears profitable to consider these (three) enlarged visceral elements in females as differences possibly associated with the larger anterior pituitaries of these females. Though we state the foregoing consideration on the

basis of observed sex differences, we note that further support for it is hardly to be found in our data dealing with racial differences; in other words, races here shown to be characterized by longer intestinal tracts have not been shown to have larger pituitaries.

TECHNIQUE AND MATERIAL

Dissection under binocular permitted the cutting of the stalk, the opening of the sella (dorsal approach), removal of the dura, and lifting out the whole gland for weighing to tenths of a milligram. The gland was then immediately returned to the moist sella where the posterior lobe was removed and the weight of the pars anterior alone thereafter obtained. The possible error was thought to be not more than 10 per cent of the weight of the posterior lobe. Many practice dissections and weighings, done to acquire speed, accuracy and uniformity in the use of this technique, were carried out before any data presented here were obtained.

Our measurement of intestinal length is for that part of the intestinal tract lying between the gizzard and the anus. Birds were killed by decapitation and soon after all intestinal movements had ceased the abdominal cavity was opened and the intestine removed. The mesenteric folds binding all curvatures were torn apart, and the intestine laid (with as nearly uniform stretching as possible) on a meter-stick for measurement. The data were obtained at all months from December to September.

The two kinds of birds studied not only belong to two different zoölogical families (pigeons, *Columba domestica*); doves, *Streptopelia risoria* and hybrids) but within each of these species several quite distinct races or racial hybrids were used. The various races of common or domestic pigeons vary greatly in size—the tipplers (*T*) being only about two-thirds as large as the homers (*H*). The ring doves used belong largely to various races which have been selectively established by one of us on the basis of thyroid size or of intestinal length. Data from each of these races or race-

hybrids have been tabulated separately. In some cases (table 1) hybrids which of course involve two races have been

TABLE 1

Average intestinal lengths and pituitary weights in males and females of various races of ring doves

RACE OR CROSS	NUMBER OF CASES	INTESTINAL LENGTH	BODY WEIGHT	INTESTINAL LENGTH BODY WEIGHT	PITUITARY		WHOLE PITUITARY BODY WEIGHT	ANTERIOR LOBE BODY WEIGHT
					Whole gland	Anterior lobe		
		<i>Cm.</i>	<i>Gm.</i>	<i>Ratio</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Ratio</i>	<i>Ratio</i>
62+	♂19	56.4	159.5	0.354	2.03	1.64	127 ¹	102 ¹
	♀15	54.3	151.2	0.359	2.00	1.61	132	106
36×62	♂19	55.1	157.8	0.349	2.04	1.66	129	105
	♀11	54.3	151.9	0.358	2.14	1.75	141	115
6+	♂14	53.3	154.4	0.345	2.11	1.69	136	109
	♀10	57.2	154.6	0.370	2.66	2.17	172	140
N2+	♂15	57.9	148.1	0.391	2.05	1.70	137	115
	♀ 9	67.0	152.7	0.439	2.22	1.83	145	120
72×63+	♂10	53.8	168.9	0.318	2.14	1.80	127	106
	♀12	55.3	158.3	0.349	2.17	1.84	137	116
72×62	♂10	53.1	168.8	0.315	2.37	1.94	140	115
	♀ 9	54.1	152.8	0.354	2.54	2.12	166	139
72+	♂10	51.1	166.9	0.306	2.42	2.00	145	120
	♀ 9	56.9	155.0	0.367	2.66	2.26	171	146
63×6+	♂ 9	50.3	156.1	0.322	2.11	1.69	135	108
	♀ 9	55.6	149.1	0.373	2.09	1.69	140	113
OT×O	♂ 9	51.3	153.3	0.335	2.03	1.63	133	106
	♀ 8	54.1	155.5	0.348	2.19	1.76	141	113
NA×72	♂ 8	48.3	153.8	0.314	2.01	1.68	131	109
	♀ 9	49.1	150.6	0.326	2.28	1.86	151	123
B+	♂10	46.2	154.5	0.299	2.18	1.85	141	120
	♀ 7	53.8	157.0	0.343	2.50	2.16	159	137
75	♂ 6	57.2	152.7	0.375	2.35	1.95	154	128
	♀ 8	58.2	135.3	0.430	2.36	1.99	175	147
51	♂ 8	49.8	153.0	0.325	1.83	1.50	120	98
	♀ 6	54.6	152.0	0.359	1.72	1.33	113	88
75×B	♂ 5	52.3	158.4	0.330	2.06	1.66	130	105
	♀ 3	50.1	150.7	0.332	2.37	2.00	151	133
Misc.	♂15	55.3	161.9	0.342	2.24	1.85	139	114
	♀13	56.2	155.4	0.362	2.25	1.83	145	118
Mean	♂(15)	52.8	157.9	0.335	2.13	1.75	135	111
Mean	♀(15)	55.4	152.1	0.365	2.27	1.88	149	124

¹ Written out fully these figures are, 0.0000127, 0.0000102, etc.

classed with one of the parent races, and to indicate that this has been done a plus (+) sign is added to the race name or number (e.g., race 62+). All birds had nearly or quite attained their adult weight. Nearly all were adult—5 to 36 months old—but about 10 per cent of both the doves and pigeons used were aged 2.5 to 3.8 months old. Our examination of pituitary size and intestinal length in this small group of younger birds shows these values to be essentially equivalent to those of adult birds of their own race, and they are therefore not tabulated separately from the adults. Only healthy birds are included, excepting that birds with light ascariasis and otherwise normal have been included. It was earlier shown by Riddle and Flemion ('28) that ascariasis induces a measurable increase in the intestinal length of ring doves; but since this infestation seems no more prevalent in one sex than in the other, and heavy ascariasis is excluded, it is probable that the inclusion of these cases (about two-fifths of total) does not decrease the accuracy of the group differences reported here.

Though some of the sex differences indicated by our data are small, statistical constants have not been calculated on this material; it is thought that the repeated occurrence of observed differences within the thirteen or fifteen races (of each of the two species studied) supplies acceptable evidence of the validity of those differences with which we are chiefly concerned.

RESULTS

In tables 1 and 2 pituitary weights and intestinal lengths of 305 healthy ring doves and 227 common pigeons are listed according to sex and race. The ring doves were of fifteen different races or race hybrids; the pigeons were of thirteen racial groups. Each of the non-hybrid races had been long inbred, was genetically different, and required treatment as a unit. For fewer than half of these races, however, is the number of measurements sufficient to give fair reliability to the values obtained.

The mean absolute weight of the whole pituitary was 5.01 mg. in male and 5.19 mg. in female pigeons; 2.13 mg. in male and 2.28 mg. in female ring doves. For anterior lobes only these weights were: 4.27 in male and 4.50 in female pigeons; 1.75 in male and 1.88 in female doves. The whole pituitary

TABLE 2

Average intestinal lengths and pituitary weights in males and females of various races of common pigeons

RACE OR CROSS	NUMBER OF CASES	INTESTINAL LENGTH	BODY WEIGHT	INTESTINAL LENGTH BODY WEIGHT	PITUITARY		WHOLE PITUITARY BODY WEIGHT	ANTERIOR LOBE BODY WEIGHT
					Whole gland	Anterior lobe		
		<i>Cm.</i>	<i>Gm.</i>	<i>Ratio</i>	<i>Mg.</i>	<i>Mg.</i>	<i>Ratio</i>	<i>Ratio</i>
M×T	♂16	83.3	316.4	0.263	4.55	3.74	144 ¹	118 ¹
	♀25	86.4	292.0	0.296	4.75	4.01	163	137
T×67	♂10	93.5	331.7	0.282	4.51	3.76	136	113
	♀16	93.7	299.5	0.313	4.79	3.98	160	132
T×33	♂15	82.8	304.6	0.272	4.49	3.82	147	125
	♀13	87.9	293.2	0.300	4.40	3.76	150	128
R×33	♂12	102.4	453.3	0.226	5.89	5.18	130	114
	♀13	95.3	391.7	0.243	5.59	4.93	143	126
T	♂10	72.4	265.2	0.273	3.99	3.30	150	124
	♀11	76.7	255.1	0.301	4.35	3.60	170	141
Compl.	♂8	104.4	387.9	0.269	5.25	4.46	135	115
	♀9	109.7	347.0	0.316	5.16	4.44	148	128
H	♂5	97.0	425.2	0.228	6.16	5.26	147	124
	♀7	98.8	436.0	0.227	6.21	5.54	142	127
H×T	♂6	94.7	350.0	0.271	4.52	3.70	129	106
	♀6	84.8	310.8	0.273	5.00	4.30	161	138
M×67	♂4	96.0	367.8	0.261	5.23	4.55	142	124
	♀7	100.1	305.6	0.328	5.43	4.67	178	153
33×67	♂5	83.6	366.6	0.228	4.70	4.12	128	112
	♀5	94.0	348.4	0.270	5.16	4.54	148	130
PT×2	♂6	88.3	314.0	0.281	5.12	4.50	163	143
	♀3	95.3	299.0	0.319	4.80	4.33	161	145
M	♂5	82.8	328.4	0.252	5.44	4.62	166	141
	♀3	87.2	295.7	0.295	6.10	5.27	206	178
67×H	♂4	85.8	403.0	0.213	5.23	4.50	130	112
	♀3	98.3	356.0	0.276	5.77	5.13	162	144
Mean	♂(13)	89.8	354.9	0.255	5.01	4.27	142	121
Mean	♀(13)	92.9	325.4	0.289	5.19	4.50	161	139

¹ Written out fully these figures are, 0.0000144, 0.0000118, etc.

of females was absolutely larger than that of corresponding males in twenty-one of the twenty-eight groups, and relative to body weight it was larger in twenty-five of the twenty-eight groups. The anterior pituitary of the females was absolutely larger in twenty, and relatively larger in twenty-seven of the twenty-eight groups. The difference between the weights of anterior lobe and whole gland represents the apparent weight of the posterior lobe; this weight of the posterior lobe, as obtained by difference, is 0.382 mg. in male and 0.395 mg. in female doves; and 0.726 mg. in male and 0.691 mg. in female pigeons. No reliable evidence of a sex difference in size of the posterior lobe has been obtained. These data, however, indicate that the posterior lobe comprises about one-sixth of the total gland (as dissected) in doves and about one-seventh in the much larger species of pigeon. It is of interest to observe that the data indicate a somewhat higher amount of pars anterior tissue per unit of body weight in the larger species (pigeons).

The females, though averaging 3.8 per cent smaller (doves), and 9 per cent smaller (pigeons), had absolutely longer intestines than the corresponding males in twenty-four of the twenty-eight races; they had relatively longer intestines in twenty-seven of the twenty-eight races—the exception being a race inadequately measured. The mean (i.e., the average of the several races) absolute intestinal length in pigeons was: males, 89.8 cm., females, 92.9 cm.; in doves, 52.8 cm. in males and 55.4 cm. in the females. The ratio of intestinal length to body weight gives an excess of 9 per cent for female doves, and of 13 per cent for female pigeons. An earlier report (Riddle and Flemion, '28) on the intestinal length of 1157 ring doves gave wholly similar results in that species.

In connection with these fully tabulated results attention should be called to a related and important point. During the past 12 years the senior author has made special effort to establish various 'endocrine races,' and success in forming races with large, small and medium size of thyroid glands

has been reported (Riddle, '29). The few pure races represented in table 1 supply strong evidence that we have also succeeded in establishing certain races with exceptionally 'long' (race N2) and others with exceptionally 'short' (races B, NA and 72) intestinal tracts. Some evidence that large and small pituitary races have also been established is supplied by these same data. For example, races 75, 72, 6, and N2 are indicated as having unusually large pituitaries (both sexes), while races 51 and 62 apparently have relatively small pituitaries in relation to body weight. Among the different races there is apparently little or no association of large pituitaries with long intestines; but the sex (female) having the largest anterior lobes has the longest intestine in (probably) all races. It is worthy of note that race 51 (with smallest pituitary found in any race) is also characterized by a body temperature more than 1°C. lower than that of any other of the twenty-four ring dove 'endocrine' races established and studied in our bird colony.

SUMMARY

For two quite different species (*Columba domestica*, *Streptopelia risoria*) it is found that the females have absolutely and relatively larger whole pituitaries and anterior lobes than have males of the same race or strain. Though the females have a smaller body weight their anterior lobes are found to average 5.4 per cent larger in domestic pigeons and 6.1 per cent larger in ring doves. Data were obtained on 305 healthy doves and on 227 pigeons.

The pars anterior of the larger species (pigeons) seems relatively larger per unit of body weight than that of the smaller species (doves).

Within these two species it is found that the females have the longer intestinal tracts. In doves the absolute excess length is 5 per cent and, in pigeons it is nearly 4 per cent; relative to body weight the excess is 9 and 13 per cent, respectively.

The data supply some evidence that races with larger and smaller pituitary size, and also races with longer and shorter intestines have been established in this colony of birds. Among the few pure races measured, however, it is not evident that pituitary size is correlated with intestinal length.

In genetically comparable adult higher vertebrates it now seems probable that larger anterior pituitaries and, possibly, longer intestinal tracts will be found associated with the female sex.

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NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

THE LONG ROAD FROM SAVAGERY TO CIVILIZATION, by Fay-Cooper Cole. 1933. Size, $5 \times 7\frac{1}{2}$ inches. Pages xi + 100. Illustrated. Bibliography. Cloth. Price, \$1.00. The Williams & Wilkins Company, Baltimore.

This volume is one of the series published in cooperation with the Century of Progress Exposition, presenting the essential features of those fundamental sciences which are the foundation stones of modern industry. The résumé of anthropology is interesting and instructive. It begins with the oldest fossils which can definitely be called human, and sketches man's physical and cultural development through the stone ages and the metal ages to the complex civilizations of Greece and Rome. It is illustrated with reproductions of paintings in the Logan Museum of Beloit College showing physical characteristics of primitive races and tribes, their manner of living, and their environment.

VERHANDLUNG DER DEUTSCHEN GESELLSCHAFT FÜR KREISLAUF-FORSCHUNG. VI Tagung. Edited by Bruno Kisch. 1933. Size, $6\frac{1}{4} \times 9\frac{1}{4}$ inches. Pages xix + 276. Illustrated with 97 text figures and 35 plates. Price, RM 15. Theodor Steinkopff, Dresden and Leipzig.

The address of the presiding officer, the lectures, articles, and discussions of the sixth session of the society, held in Wurzburg, March 6 and 7, 1933. Many of the papers were concerned with phases of the general subject of circulation and the nervous system. The discussion of theoretical questions was led by Dr. H. E. Hering, and the discussion of clinical problems by Dr. G. Kauffman.

